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The Impact of Anaesthesia Protocols on BOLD fMRI Validity in Laboratory Rodents – a Systematic Review

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Table of Contents

Abstract

BOLD fMRI is widely used for neuroimaging in rodent models and typically requires anaesthesia. Here, the literature investigating the influence of different anaesthesia regimes and changes in physiological parameters as confounders of BOLD fMRI in rats and mice was systematically reviewed, following a published protocol for systematic reviews of animal studies. Four databases were searched. 122 publications met predefined inclusion criteria. Dose rates of anaesthetics and timing of image acquisition relative to induction of anaesthesia commonly influenced readouts under otherwise identical conditions. The heterogeneity of included studies prevented strong conclusions for pairwise comparison of anaesthetics. Differences between awake and anaesthetized imaging were however consistently reported. Arterial blood pressure, arterial partial pressure of oxygen and carbon dioxide affected BOLD signal across various experimental paradigms and should therefore be maintained at stable physiological levels throughout experiments. Despite high risk of bias in all included studies, this review demonstrates that BOLD fMRI studies risk to be confounded by anaesthetic protocols and management. Establishing evidence-based optimal dose ranges and imaging timepoints for a selection of anaesthetic protocols, as well as standards of anaesthetic monitoring should therefore be a priority for improving the validity of BOLD fMRI in anaesthetised rodent models.

Keywords: BOLD fMRI, anaesthesia, monitoring, rat, mouse

Zusammenfassung

Neurologische Bildgebung mittels BOLD fMRI erfolgt bei Nager-Modellen üblicherweise unter Anästhesie. In der vorliegenden Arbeit wurde die Literatur, welche den Einfluss verschiedener Anästhesieprotokolle und Veränderungen physiologischer Parameter als Störfaktoren auf BOLD fMRI bei Ratten und Mäusen untersucht, in einem systematischen Review zusammengefasst. 122 Publikationen aus vier Datenbanken erfüllten die zuvor definierten Einschlusskriterien. In den meisten Fällen beeinflussten die Dosisraten der Anästhetika sowie der Zeitpunkt der Bildgebung bei sonst gleichen Bedingungen die Ergebnisse. Die Heterogenität der eingeschlossenen Studien erlaubte keine aussagekräftigen Schlussfolgerungen für Vergleiche zwischen Anästhetika. Unterschiede zwischen Messungen bei wachen und anästhesierten Tieren wurden jedoch übereinstimmend beschrieben. Arterieller Blutdruck, arterieller Sauerstoff- und Kohlenstoffdioxidpartialdruck beeinflussten das BOLD Signal und sollten während des Experiments auf physiologischem Niveau stabilisiert werden. Trotz des hohen Risikos für Bias in allen Studien zeigt diese Arbeit, dass BOLD fMRI Studien für verzerrende Einflüsse von Anästhesieprotokollen anfällig sind. Evidenzbasierte optimale Dosisbereiche und Bildgebungs-Zeitfenster

für eine Auswahl von Protokollen sowie Standards für die Anästhesieüberwachung sollten etabliert werden, um die Validität von BOLD fMRI Studien bei anästhesierten Nager-Modellen zu verbessern.

Stichworte: BOLD fMRI, Anästhesie, Monitoring, Ratte, Maus

1 Introduction

1.1 BOLD fMRI for functional neuroimaging

Blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) is the most commonly used functional neuroimaging modality in humans and widely used in preclinical and basic research rodent models (Martin, 2014; Jonckers et al., 2015; Pan et al., 2015). Changes in blood oxygenation levels are interpreted as a surrogate for neuronal activation, based on the mechanism of neurovascular coupling (Logothetis and Pfeuffer, 2004). Upon neuronal activation, a feedforward mechanism dilates arterioles and potentially also capillaries to allow more fully oxygenated blood to flow in (Attwell et al., 2010). As the increased supply of oxygen (O_2) exceeds the increase of O_2 consumption associated with neuronal activation, venous oxygenation increases in those areas. The BOLD signal arises from changes of deoxyhaemoglobin content per unit of brain volume. Deoxyhaemoglobin content is a function of cerebral metabolic rate of oxygen ($CMRO_2$), cerebral blood flow (CBF) and cerebral blood volume (CBV) (Kim and Ogawa, 2012).

1.2 BOLD fMRI and anaesthesia

As fMRI is susceptible to movement artefacts, animals need to be immobilised for image acquisition. Traditionally, this has been achieved by general anaesthesia and while imaging of conscious animals is gaining popularity (Gao et al., 2017), it is still common practice to image rats and mice under general anaesthesia or sedation. In terms of animal welfare, scanning under anaesthesia is preferable to awake scanning under rigorous fixation (Low et al., 2016).

However, anaesthesia drug- and dose-dependently modulates aspects of neurovascular coupling: first, neuronal baseline metabolism and thus $CMRO_2$ is markedly reduced compared to the awake state (Gao et al., 2017). Second, anaesthetics may modulate the signal cascades responsible for neurovascular coupling on the molecular level (Nakao et al., 2001; Petzold and Murthy, 2011). Third, haemodynamic baseline conditions and vascular reactivity are typically altered, either as a result of direct drug effects on cerebral vasculature or as a result of systemic cardiovascular and respiratory depression. CBV and CBF responses to stimulation are typically slower and have a lower amplitude in anaesthetised animals compared to conscious animals (Gao et al., 2017). For example, hypotension below autoregulatory limits reduces cerebral perfusion pressure (if intracranial pressure remains constant) and may thus reduce the CBF response. Similarly, hypoventilation in spontaneously breathing animals typically results in elevated partial pressure of carbon dioxide (pCO_2), which induces vasodilation and thus limits maximal vasodilation in response to stimuli.

Beyond the impact of anaesthetics on neurovascular coupling and the resulting potential for bias in signal detection (false positives and negatives), anaesthetics alter neuronal activation and information processing so that measurements may reflect

activation or spontaneous activity under a distinct state of anaesthesia rather than universal patterns.

While it is very likely that the choice of a specific anaesthetic protocol will have an impact on the observed BOLD signal, predicting the overall effect of all potentially involved mechanisms is almost impossible. A systematic review of anaesthetic protocols used for phMRI found a wide variety of agents, combinations, dosages and respiratory gases used (73 different protocols in 126 studies) (Haensel et al., 2015). Knowing the “profile” of effects of different drugs and drug combinations on the BOLD signal is vital for the integration of findings across studies as well as for planning further investigations. Although studies have measured how different anaesthetics, dosages or timepoints of imaging affect the BOLD signal acquired under otherwise identical imaging paradigms, a systematic review of those findings has not been published yet. Similarly, alterations in systemic physiological parameters are common under general anaesthesia and sedation and can induce bias in BOLD fMRI experiments if they are not monitored, but a consensus on minimal monitoring for rodent BOLD fMRI experiments has not been established.

1.3 Aim of the review

One part of this review therefore focuses on studies which directly compare different anaesthetic protocols with each other or with awake scanning. We want to analyse how different states of anaesthesia affect the BOLD outcome measures specified in the respective studies in adult rats and mice. The other part of this review focuses on the specific effects of alterations in systemic physiological parameters on different BOLD outcome measures.

Our aim is to demonstrate the extent of anaesthetic protocol-related differences in BOLD fMRI outcomes, to formulate evidence-based minimal standards for monitoring during BOLD fMRI experiments, and ultimately to elaborate recommendations on how to choose an appropriate anaesthetic protocol. To our knowledge, this is the first systematic review about the impact of anaesthetic protocols on BOLD fMRI validity in laboratory rodents.

The concept of systematic reviews was originally developed for clinical studies (Moher et al., 2000) and recently extended and adapted to pre-clinical studies (de Vries et al., 2015). The question we are addressing is however from the field of basic research. While narrative reviews are commonly encountered in basic research, systematic reviews are not established yet. This systematic review therefore also serves as a case-study for the application of systematic reviews in basic research.

2 Materials and Methods

This review was designed as a systematic review. The „Systematic review protocol for animal intervention studies" (de Vries et al., 2015) was used for preparation and validation. The protocol as on the day when the systematic literature search was performed is provided in appendix 2.

2.1 Systematic literature search

A systematic search was conducted in Embase, Medline, Scopus and Web of Science in august 2017. Search terms are listed in table 1. Each reference had to contain at least one term of each building block. Terms were searched in titles and abstracts (Medline, Embase) or the closest option available (title, abstract, keywords for Scopus and Web of Science). Emtree vocabulary and MeSH were additionally used for the search in Embase and Medline, respectively, if a correspondent entry existed. Language was restricted to English, German and French. As the first article describing BOLD contrast in MRI was published in 1990, filters for publication year 1990 or later were used.

Table 1. Structure of systematic literature search. Search terms within one building block were linked with "OR", building blocks with "AND". Database-specific syntax, truncation options and proximity operators ("NEAR" for Embase and Web of Science, "adj" for Medline, "W" for Scopus) were used.

Rodents
rat OR rats OR mouse OR mice OR rodent OR rodents
MRI
((MRI OR MRT OR NMR OR "magnetic resonance imaging") *proximity operator*5 functional) OR fMRI OR BOLD OR "Blood oxygen level dependent"
Anaesthesia OR physiology
anesthe* OR anaesthe* OR hypercapnia OR hyperoxia OR hypoxia OR apnoea OR "blood pressure" OR hypotension OR hypertension OR autoregulation OR thermoregulation OR "physiological noise" OR "functional connectivity" OR somatosensory OR stimulation OR isoflurane OR sevoflurane OR halothane OR medetomidine OR dexmedetomidine OR alpha-chloralose OR chloralose OR α-chloralose OR urethane OR propofol OR ketamine OR xylazine

2.2 Screening and study selection

Systematic review software, DistillerSR (Evidence Partners, Ottawa, Canada), was used for screening, study selection and later data extraction. References were automatically checked for duplicates before entering the screening process. For the screening process, a refined version of the eligibility criteria formulated in the study protocol was used (appendix 3).

Studies were eligible if they reported BOLD fMRI results of the brain of adult rats or mice under different anaesthetic or physiologic conditions. Differences in physiological parameters could be intentionally induced (interventional studies) or naturally occurring, but explicitly analysed for their effect on BOLD signal (observational studies; intention to analyse effects of physiological parameter fluctuations on BOLD signal had to be expressed in abstract). The publication had to describe original research. Reviews, book chapters and opinion pieces were excluded. Re-analysis of previously acquired data, however, was eligible as a strategy to reduce the number of animal experiments. Full articles were included as well as short forms (conference papers/abstracts/posters). No restrictions regarding sex, strain or health status of the animals were imposed. Adult was defined as at least 8 weeks of age or 18 g for mice and at least 8 weeks or 200 g for rats and up to 12 months of age for both species. If age or weight ranges at the lower end of the scale “overlapped” with the definition of adult, so that it was to be expected that the majority of animals met the inclusion criteria (e.g. rats of 180 to 240 g), the study was included. Studies which did not report age or weight of the animals were included if they not explicitly stated that younger (e.g. pups, neonatal, juvenile, adolescent) or older (geriatric, aged) animals were investigated.

BOLD fMRI had to be applied to the brain. Studies imaging other regions of the body (e.g. spine, heart, kidneys) or brain tumours were excluded. Other fMRI modalities such as arterial spin labelling (for CBF measurement) or contrast enhanced CBV measurements and structural MRI studies were not eligible. Whether imaging was performed under stimulation (peripheral/central), as a pharmacological MRI (phMRI) or in the resting state, was irrelevant for inclusion. Similarly, no restrictions were made regarding outcome measures except that they had to be directly derived from the BOLD signal. Correlations of the BOLD signal with signals from other functional neuroimaging methods, with measurements of neural activity or cerebral haemodynamics were however excluded. In the special case of studies determining $CMRO_2$ with multimodal fMRI, the decision was based on whether the BOLD signal was separately reported or not. If a study used multiple methods (e.g. EEG and BOLD fMRI), only data from those experiments meeting the inclusion criteria were analysed.

Other reasons of exclusion were duplicates which were not recognised by the automatic screening, short forms for which a corresponding full article was available and publications with a strong suspicion of multiple reporting (criteria: same authors, same number of animals in same experimental design, similar methods of analysis/same outcome measure and no declaration that data from a previous study was re-analysed).

For included short forms, Embase and Google Scholar were searched for publications of the same authors within 3 years of publication of the short form in order to identify corresponding full articles.

Any reference fulfilling inclusion criteria, but not identified by the systematic search, was included if found in the literature.

Study selection was performed by a single reviewer. Currently there is not enough evidence to claim independent screening by two reviewers (Shamseer et al., 2015). The screening process consisted of two stages. In the first stage (title abstract screening), title and abstract were screened for obvious exclusion reasons such as other species, imaging of other body regions, absence of fMRI, or reviews and opinion pieces. Any publication using BOLD fMRI was included at this stage. If it was not clear from the abstract which (f)MRI modality was used or with which method functional connectivity (fc) was measured, the reference was included. Similarly, references for which no abstract was displayed were taken to the next level unless the title clearly indicated that they investigated other species or body regions. For the second screening stage (full text screening), full text versions of all references were acquired. Based on the full text it was decided whether a publication was in- or excluded. References for which this decision was not straightforward are listed in appendix 4 together with a rationale.

2.3 Data extraction

In a quarter of included studies, data were extracted in double by a second reviewer (Frédérik Rousseau-Blass) to ensure consistency of data extraction. For each included study, animal characteristics (species, sex, strain, age, weight, numbers per experimental group), the exact anaesthetic protocol (drugs, doses, route of administration, time point of administration, gas mixture, gas flow, concentration of inhalant anaesthetic) and presence or absence of monitoring of specific parameters (temperature, heart rate, arterial oxygen saturation (SpO_2), respiratory rate/ventilator setting, end-tidal gas concentrations, blood gas parameters and timing, arterial blood pressure, reflexes) were extracted. Additionally, surgical preparations for the experiment, study design, type and timing of stimulations during BOLD fMRI, duration of image acquisition and total experiment, magnetic field strength, regions of interest and the data analysis approach were extracted. For records investigating the effect of physiological parameters on BOLD fMRI outcome, details of the interventions to alter the physiological parameter(s) and the observed changes in physiological parameters were additionally extracted.

Forms for data extraction were implemented in DistillerSR and are available in appendix 7.

To account for the variety of outcome measures used in individual studies, the outcome to be extracted from each study was defined as whether a – qualitative or quantitative – difference in the individual outcome measure was observed between different states of anaesthesia or physiologic conditions. If a difference was observed, it was specified.

2.4 Data analysis

Data was analysed in a structured (narrative) synthesis. Data was analysed for rats and mice separately but following the same structure algorithm.

For the comparison of physiological conditions, references were grouped by experimental paradigm (e.g. resting state fMRI (rsfMRI), fMRI measuring response to a certain type of stimulation). If a reference reported results for multiple experimental paradigms, it was allocated to all paradigms for which inclusion criteria were fulfilled. Within each experimental paradigm we analysed whether in- and/or decreases of specific physiological parameters were consistently reported to affect BOLD outcome measures, and whether the reported effects were consistent, complementary or inconsistent.

For the comparison of states of anaesthesia, analysis followed an analogous structure. References were grouped by experimental paradigm (e.g. rsfMRI, fMRI measuring response to a certain type of stimulation). If a reference reported results for multiple experimental paradigms, it was allocated to all paradigms for which inclusion criteria were fulfilled. Within each experimental paradigm we analysed whether studies comparing the same anaesthetics, different doses of the same anaesthetic or different timepoints of imaging for the same anaesthetic, consistently reported differences, and whether the observed differences were consistent, complementary or inconsistent.

The order in which comparisons would be addressed per paradigm as well as to which level stimulation paradigms would be differentiated was not a priori defined in order to allow clustering based on the included publications.

2.5 Risk of bias assessment

Risk of bias was assessed for individual studies using an adapted version of the SYRCLE risk of bias tool (Hooijmans et al., 2014), to our knowledge the only standardised tool for the assessment of risk of bias in animal intervention studies. Signalling questions and examples provided for each item were adapted to the settings of the studies under investigation. Item number 4 of the original tool, randomised housing, was excluded because preliminary literature search suggested that many BOLD experiments are performed in one session without prior interventions whose effects could vary due to different housing conditions. Moreover, while it is plausible that differences in individual experiences (social status, cage position in the room) have an impact on the brain’s fc and reactivity to specific stimuli, such variance first adds to external validity of a study and it is second not known, whether and how such interindividual differences are transmitted to the anaesthetised or sedated state.

The adapted version of the tool can be found in appendix 6. To ensure consistent assessment of studies, rules derived from specific examples were defined and continuously updated. Individual studies were assessed as having a low, unclear or high risk of bias according to the Cochrane Collaboration’s tool for assessing risk of bias in randomised trials (Higgins et al., 2011).

High risk of bias was not an exclusion criterion. Instead, risk of bias across studies was indicated for each subgroup of analysis and resulted together with the consistency of the presence and direction of effects in an assessment of the strength of evidence for that particular subgroup (Berkman et al., 2015).

3 Results

3.1 Systematic literature search

A total of 9984 references was retrieved by the systematic literature search and 6284 remained after duplicate removal. Of those, 4875 references were excluded at the first screening level and 1289 at the second screening level. Finally, 122 references were included, two of which were not found with the systematic search but forwarded by a colleague.

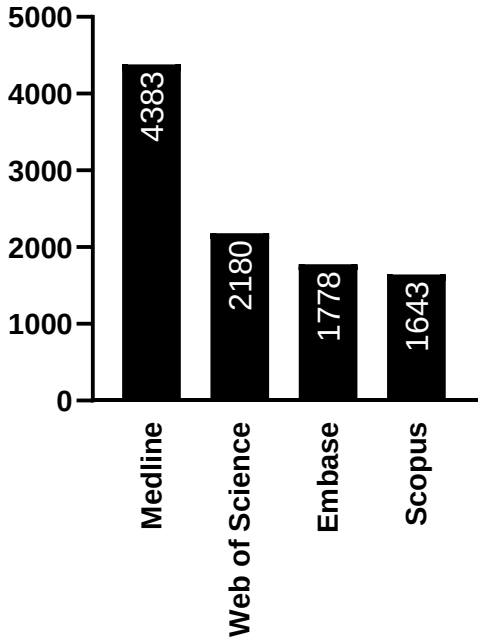

Figure 1: Number of references found via systematic literature search, grouped by database.

The predefined inclusion and exclusion criteria did not cover all constellations encountered. During study selection, additional exclusion criteria were defined:

Interventions tested on one animal only were excluded. References investigating the effects of physiological parameters were excluded if the anaesthetic agent was not reported. The results section had to contain at least one sentence about the comparison of interest. In selected cases, which are justified in appendix 4, references were excluded for insufficient detail of the reported results. As a rule of thumb, studies presenting only quantitative findings such as percent signal changes

without a statement on significance were excluded. Descriptive reporting of qualitative aspects was accepted unless generalised to the point that almost no information could be extracted (e.g. one sentence about “widespread activation” without further characterisation of the location, extent or reproducibility of that activation compared to the second experimental condition).

Some studies used known effects of hypercapnia or hyperoxia to study differences in response to hyperoxic or hypercapnic challenges between experimental groups, without further characterising those responses, and were excluded. Two studies were excluded because animals were imaged in a vertical position. As orthostatic arterial blood pressure regulation is blunted during anaesthesia (Duke-Novakovski et al., 2016), the haemodynamic status of vertically positioned animals was not considered comparable to the haemodynamic status of animals in horizontal position.

Additional specific cases are described in appendix 4. The search also retrieved a few abstract collections or conference proceedings, typically containing several hundred abstracts. Those were excluded with the reasoning that individual potentially relevant abstracts could have been identified by the systematic search and are also listed in appendix 4.

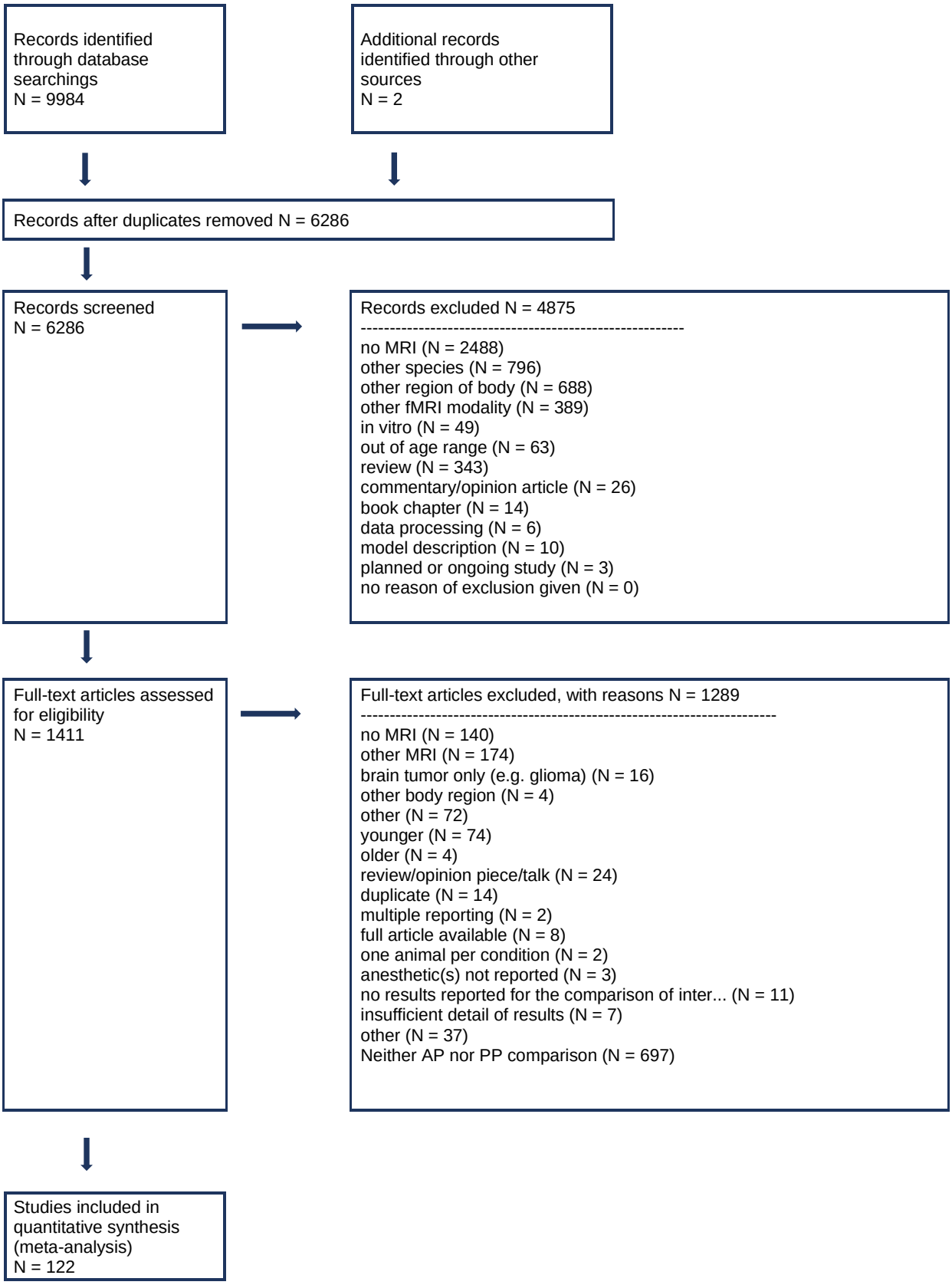
Records identified through database searchings N = 9984
Additional records identified through other sources N = 2
Records after duplicates removed N = 6286
Records screened N = 6286
Records excluded N = 4875
no MRI (N = 2488)
other species (N = 796)
other region of body (N = 688)
other fMRI modality (N = 389)
in vitro (N = 49)
out of age range (N = 63)
review (N = 343)
commentary/opinion article (N = 26)
book chapter (N = 14)
data processing (N = 6)
model description (N = 10)
planned or ongoing study (N = 3)
no reason of exclusion given (N = 0)
Full-text articles assessed for eligibility N = 1411
Full-text articles excluded, with reasons N = 1289
no MRI (N = 140)
other MRI (N = 174)
brain tumor only (e.g. glioma) (N = 16)
other body region (N = 4)
other (N = 72)
younger (N = 74)
older (N = 4)
review/opinion piece/talk (N = 24)
duplicate (N = 14)
multiple reporting (N = 2)
full article available (N = 8)
one animal per condition (N = 2)
anesthetic(s) not reported (N = 3)
no results reported for the comparison of inter... (N = 11)
insufficient detail of results (N = 7)
other (N = 37)
Neither AP nor PP comparison (N = 697)
Studies included in quantitative synthesis (meta-analysis) N = 122

Figure 2. Prisma flow chart representing workflow from reference identification to definitive inclusion. The category “other” at the full-text screening level contains all reasons of exclusion that were not previously mentioned, such as quantitative fMRI not reporting BOLD signal changes, application of hypercapnia or hyperoxia, correlation of the BOLD signal with other parameters as the sole outcome measure, and less common reasons specified in appendix 4. Flow chart generated with DistillerSR (Evidence Partners, Ottawa, Canada), based on (Moher et al., 2009).

3.2 Data structure

Of the 122 included references, 117 were full articles and 5 short forms of publications such as conference abstracts or posters. Those 122 publications were based on minimally 112 datasets. The exact number of datasets is difficult to identify because a cluster of five publications (Liang et al., 2012a, b; Liang et al., 2013; Liang et al., 2015a; Smith et al., 2017) appeared to re-analyse the same core dataset (16 rats imaged awake and under isoflurane), adding however data from variable numbers of awake animals acquired in one of those five studies (Liang et al., 2012a) or in a previous study (Zhang et al., 2010). The reference cited for the core dataset (Liang et al., 2011) is not included in this review because findings from anaesthetised animals are not reported in that publication. Due to the intransparency of data provenance, those five references were considered as based on one dataset for further analyses.

Unless indicated otherwise, the number of references corresponds to an equal number of datasets in the following section. In chapters 3.3 and 3.4, results are first reported for individual references and then summarised per dataset for the conclusions.

Rats were studied in 107 references based on 99 datasets and mice in 15 references based on 13 datasets; no publication reported results for both species.

The effect of changes in physiological parameters on the BOLD signal was addressed in 45 rat references (45 datasets) and 4 mouse references (4 datasets). Differences in BOLD fMRI measurements between anaesthetic protocols and/or awake and anaesthetised animals were addressed in 70 rat references (62 datasets) and 14 mouse references (12 datasets). Eight and three references in rats and mice, respectively, covered the effects of physiological parameters as well as anaesthetic protocol comparisons.

Risk of bias was assessed as high in all included references. Lack of blinding, both during the experiment (121/122 references clearly not blinded, 1 reference unclear) and during data analysis (119/122 studies clearly not blinded, 4 studies unclear), was the primary reason for this classification, followed by concerns associated with study design (29/122 references, including the cluster of 5 references discussed earlier and 4 references based on 2 datasets; i.e. 22/112 datasets).

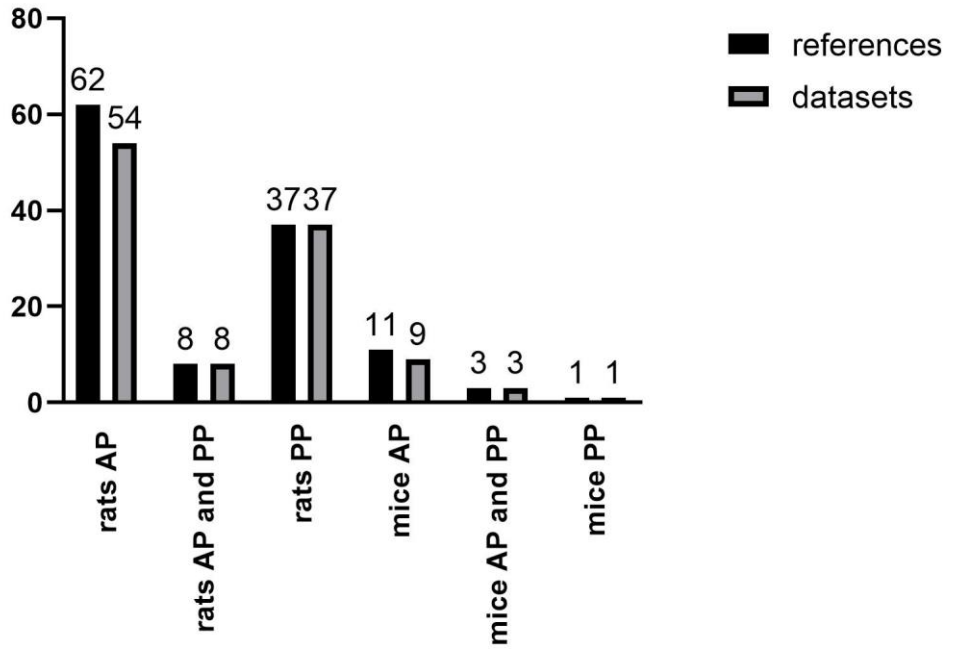

Figure 3. Numbers of included references and underlying datasets per species and category. AP = comparison of states of anaesthesia, PP = investigation of effects of physiological parameters, AP and PP = comparison of states of anaesthesia as well as investigation of effects of physiological parameters.

Included studies were heterogeneous in terms of animal characteristics, study design, experimental procedures and image acquisition.

Sprague-Dawley rats were the most commonly used rat strain (57 references/55 datasets, of which 1 also used Wistar rats), followed by Wistar (31 references/30 datasets, of which 1 also used Sprague-Dawley rats) and Long-Evans rats (17 references/12 datasets). In three studies the rat strain was not specified. All mouse studies used C57BL/6 mice, and two studies additionally investigated other strains (BALB/c and I/LnJ in one study, transgenic animals expressing channel rhodopsin 2 in the other study). While primarily male rats were studied, both sexes were represented in mouse studies (figure 4).

Animal numbers for fMRI ranged from 2 to 55 rats and from 8 to 63 mice per publication. Most studies either exposed different groups of animals to different conditions or successively exposed the same animals to multiple conditions in a single experimental session. Combinations of the previously mentioned designs and exposition of the same animals to different conditions in different experimental sessions were also represented (figure 5).

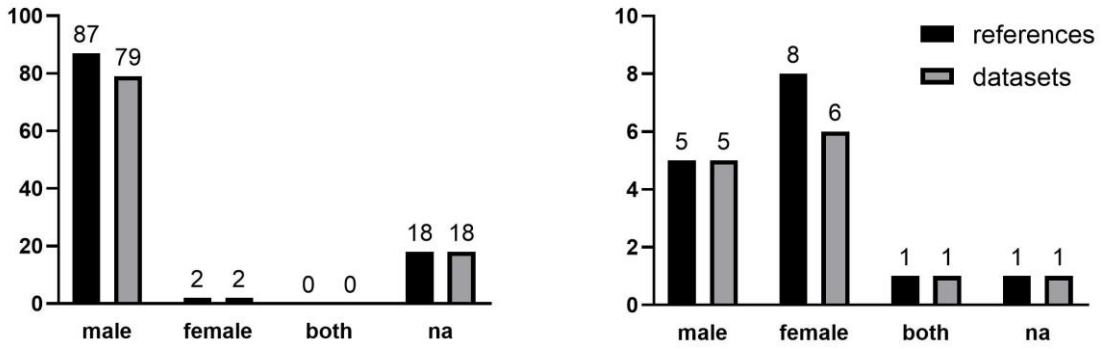

Figure 4. Sex distribution in studies investigating rats (left diagram) and mice (right diagram). Numbers of references and underlying datasets are shown. Male = study used only male animals, female = study used only female animals, both = study used animals of both sexes, na = sex of animals not reported

Experimental procedures, for example whether animals were intubated and/or mechanically ventilated, or whether the same anaesthetic was used for induction and maintenance of anaesthesia, varied between studies, as did anaesthetic monitoring and total experimental times. A few studies performed surgical procedures on the head directly before fMRI acquisition (intracerebral or epidural electrode placement in nine references, skull exposure in two references, middle cerebral artery occlusion in one reference, implantation of a head bar plus a gastric tube in one reference). Tracheotomy was reported in roughly a quarter of included references (32, including 4 that also did surgery on the head).

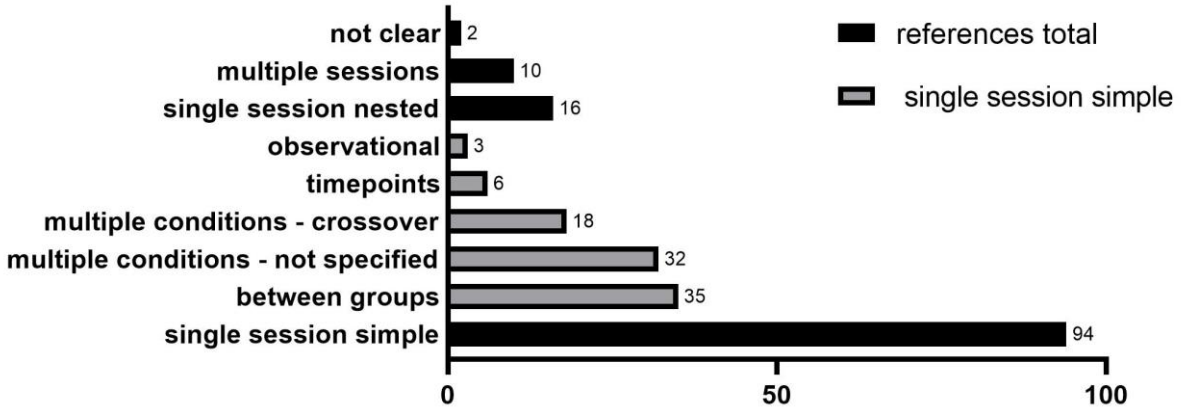

Figure 5. Study designs used in included references. Reference counts are presented, because some references re-analysed only parts of the original dataset. Not clear = the study design was not clear from the provided information; multiple

sessions = animals underwent several experimental sessions on different days; single session nested = elements of simple study design are combined; single session simple = study design can be described by one of the following categories: between group = one group of animals per condition, one condition per animal; multiple conditions – not specified = multiple conditions per animal in not specified or fixed order; multiple conditions – crossover = multiple conditions per animal in a crossover design; timepoints = one condition per animal, multiple measurements at different timepoints; observational = studies measuring and analysing the effect of naturally occurring signal fluctuations on BOLD signal.

Heterogeneity between studies may further arise from image acquisition, processing and analysis. Magnetic field strength of scanners used in the included studies varied from 1.5 T to 11.7 T and was most commonly between 4.7 and 9.4 T (figure 6). Assessing additional technical specifications, the methods used for image processing and approaches to data analysis was however beyond the scope of this review.

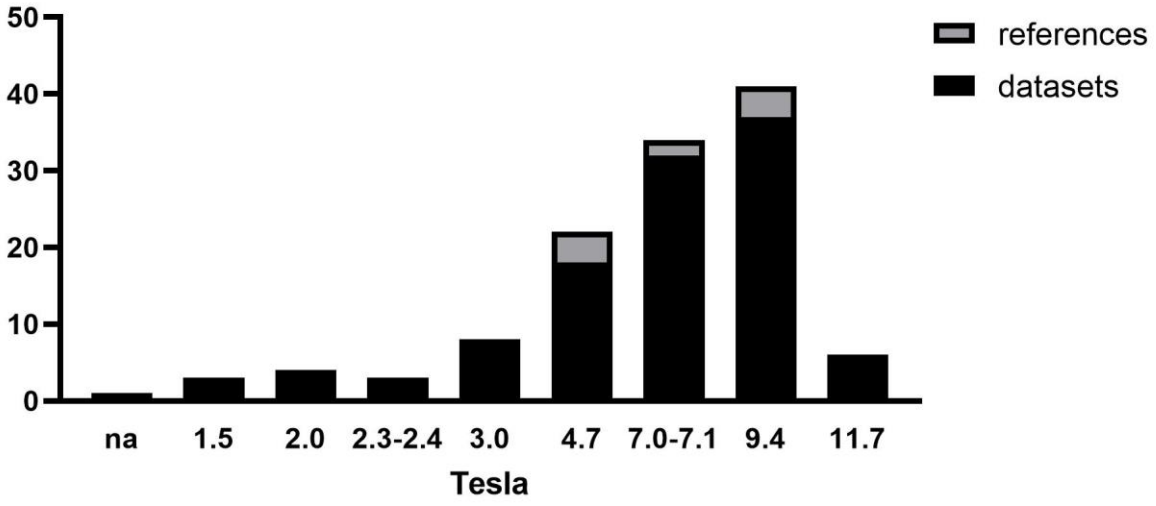

Figure 6. Magnetic field strength in tesla of MRI scanners used in the included studies.

3.3 Effects of physiological parameters on BOLD signals

3.3.1 Rats

3.3.1.1 Effects of changes in physiological parameters on the baseline BOLD signal

The impact of alterations in systemic physiological parameters on the baseline BOLD signal was investigated in 31 interventional and 1 observational references. 27 did so under one anaesthetic condition, 5 (interventional) compared different conditions. Whether findings depended on anaesthetic protocols will be covered in chapter 3.4.

The majority of references changed the concentrations of inspiratory gases and/or induced apnoea.

3.3.1.1.1 Hypoxaemia and concomitant hypoxia

Eleven studies investigated the effect of hypoxic gas mixtures on BOLD signal and consistently report a decrease of baseline BOLD signal intensity or an increase of effective relaxation rate (R2*), which translates into lower signal intensity, under varying degrees of hypoxia and hypoxaemia. This effect was found across a range of anaesthetic protocols (halothane, isoflurane, pentobarbital, α-chloralose, urethane, ketamine/xylazine, awake) and under spontaneous (Prielmeier et al., 1993; Dunn et al., 1999; Sicard and Duong, 2005; Duong, 2007) as well as controlled ventilation (Prielmeier et al., 1994; Kida et al., 1996; Lin et al., 1998b; Lin et al., 1998c; Houston et al., 2000). Sicard and Duong (2005) observed lower baseline BOLD signals with more pronounced hypoxia. A linear relation between the change in R2* (ΔR2*) and venous or weighted arterial plus venous O_2 saturation was commonly observed (Prielmeier et al., 1994; Kida et al., 1996; Lin et al., 1998b; Lin et al., 1998c). One study found that the slope of this linear relationship differs significantly between normal and reduced haematocrit, with steeper slopes in anaemic animals (Lin et al., 1998b). Signal intensity was typically reduced in vessels (e.g. sagittal sinus) and brain parenchyma, but some studies did not differentiate (e.g. Lowry et al. (2010). While studies consistently reported stronger (Prielmeier et al., 1993; Prielmeier et al., 1994) or earlier (Kida et al., 1996) reductions of signal intensity or increases of R2* close to vessels in comparison to brain parenchyma, regional differences within brain parenchyma were more controversial. Houston et al. (2000) mention regional differences in signal decrease without further specifying them. Prielmeier et al. (1993) report higher signal changes in cortex than in caudate putamen (CPu) under halothane, whereas Dunn and Swartz (1997) observed a higher R2* in the CPu than cortex (i.e. opposite trend) under ketamine/xylazine. However, it is not clear whether those differences were statistically significant. Dunn et al. (1999) found significantly lower ΔR2* in hippocampus than in cortex and thalamic nuclei, while corpus callosum values were in between and not significantly different from other regions. Finally, Kida et al. (1996) noted that the decrease in signal intensity became evident in cortex prior to basal ganglia when the fraction of inspired oxygen (F_iO_2) was reduced stepwise , but Prielmeier et al. (1994) did not find a significant difference in ΔR2* between cortical and basal ganglia region of interest (ROI) .

Taken together, there is robust evidence that BOLD signal intensity decreases under hypoxic conditions. Some degree of regional heterogeneity in BOLD signal decrease is likely to occur, but whether it reaches statistical significance and where the

strongest and weakest changes have to be expected cannot be concluded from the current evidence.

3.3.1.1.2 Hyperoxaemia and hyperoxia

Three studies consistently report an increase in BOLD signal intensity during hyperoxia in healthy animals under controlled as well as spontaneous ventilation (Sicard and Duong, 2005; Lu et al., 2009; Lowry et al., 2010). Lu et al. (2009) observed strong signal changes primarily “in the outer layer of the cortex and several subcortical areas” including CPu and thalamus, the other two studies did not investigate regional differences. A fourth study used hyperoxia as a challenge in a stroke model and found that both 40 and 100% inspiratory O_2 significantly increased effective transverse relaxation time (T2*), i.e. BOLD signal intensity, in the penumbra of an ischemic lesion compared to the ischemic core as well as the contralateral unaffected cortex (Baskerville et al., 2011). While in the latter two, changes in signal intensity did not significantly differ between 40 and 100% O_2, in penumbra, signal increase under 100% O_2 was significantly higher.

In contrast, a fifth study briefly reports that no significant difference was found in baseline BOLD signal between animals ventilated with room air and 100% O_2 under pentobarbital anaesthesia (Kannurpatti et al., 2003a).

In summary, an increase in BOLD signal intensity due to hyperoxia was reported in some references (Sicard and Duong, 2005; Lu et al., 2009; Lowry et al., 2010; Baskerville et al., 2011) but not in earlier work (Kannurpatti et al., 2003a).

3.3.1.1.3 Hypercapnia

Six studies consistently report an increase in BOLD signal intensity during hypercapnia in awake as well as isoflurane-, nitrous oxide (N_2O)- or pentobarbital-anaesthetised animals and under both spontaneous and controlled ventilation (Graham et al., 1994; Brevard et al., 2003; Kannurpatti et al., 2003a; Sicard et al., 2003; Sicard and Duong, 2005; Lu et al., 2009). A trend for higher signal increases under higher carbon dioxide (CO_2) concentrations (2 versus 5% and 5 versus 10%) was consistently observed (Brevard et al., 2003; Kannurpatti et al., 2003a; Sicard et al., 2003; Sicard and Duong, 2005), but only in one study the difference reached statistical significance (Kannurpatti et al., 2003a). Most studies investigated whole brain ROI, only Lu et al. (2009) differentiated between different ROI and reports significant signal increases in cortex, thalamus, and depending on whether gradient echo or spin echo was used also in CPu, but not in hippocampus. Within the cortex signal changes were primarily found in the middle layer (2 mm below surface). An additional study repeatedly acquired scans 5 min after termination of 5 min of hypercapnia and did not find a significant increase of baseline BOLD signals, which was in line with a return of arterial partial pressure of CO_2 (p_aCO_2) to pre-hypercapnia levels (Dutka et al., 2002).

Taken together, there is robust evidence that hypercapnia transiently increases BOLD signal intensity across a range of conditions.

3.3.1.1.4 Hyperoxia/hypercapnia

One single report of combined hyperoxia and hypercapnia observed higher baseline BOLD signal intensity under carbogen, i.e. 95% O_2 plus 5% CO_2, than under room air (Kannurpatti et al., 2003a).

3.3.1.1.5 Apnoea

Three studies imposed apnoea on animals ventilated with room air, hyperoxic, hypercapnic or combined hyperoxic/hypercapnic gas mixtures under pentobarbital or urethane anaesthesia.

In animals ventilated with room air, BOLD signal consistently decreased across all brain regions during apnoea (Kannurpatti et al., 2003a, b; Kannurpatti and Biswal, 2004). When the duration of apnoea was increased from 20 to 30 s, a significant signal decrease was observed in more voxels and the magnitude of signal decrease tended to be higher. Regardless of the duration of apnoea, signal decrease was significantly larger in cortex than thalamus and hippocampus (Kannurpatti et al., 2003b).

When animals were ventilated with 100% O_2, BOLD signal increased during apnoea (Kannurpatti and Biswal, 2004; Kannurpatti et al., 2003a). Distribution of the signal change was similar as for the room air condition, but reportedly fewer voxels were affected (no information on significance provided).

Ventilation with 2 or 5% CO_2 added to room air resulted in a significantly higher signal decrease than in the room air condition (Kannurpatti et al., 2003a; Kannurpatti and Biswal, 2004).

The combined effects of hyperoxic and hypercapnic inspiratory gases appeared to level out when animals were ventilated with carbogen, i.e. a mixture of 95% O_2 and 5% CO_2: no significant change in BOLD signal was detected during apnoea (Kannurpatti et al., 2003a).

Taken together, a global change of BOLD signal intensity during apnoea can be expected for most inspiratory gas compositions, although the direction and magnitude of the signal change will depend on the inspiratory gas composition.

3.3.1.1.6 Hypotension or decrease in arterial blood pressure

Four studies investigated the effect of arterial blood pressure decreases on the baseline BOLD signal. Arterial blood pressure decreases were induced by blood withdrawal (Zaharchuk et al. (1999) halothane, Kalisch et al. (2001) halothane, isoflurane, propofol) or pharmacologically (Wang et al. (2006) α-chloralose) in three studies. The fourth study analysed potential correlations between blood pressure and BOLD signal intensity under different isoflurane concentrations (Tsurugizawa et al., 2016).

Zaharchuk et al. (1999) did not find significant changes in R2*, neither in a whole brain slice ROI, nor when separately analysing striatum, cortex and cortical rim. In contrast, Kalisch et al. (2001) observed significant positive correlations between arterial blood pressure and BOLD signal intensity in an entire brain slice, however,

correlations were region-specific with larger positive and negative (> 0.5 / < -0.5) correlations in the proximity of large vessels, positive correlations in cortex and some subcortical clusters, and negative correlations in other clusters. Contrary to the mixed positive and negative correlations of Kalisch et al. (2001), Wang et al. (2006) uniformly found a decrease in BOLD signal when arterial blood pressure decreased. The number of arterial blood pressure-correlated voxels depended linearly and significantly on the magnitude of arterial blood pressure change and became substantial for changes exceeding 30 mmHg. Those voxels were distributed across cortical and subcortical areas.

Tsurugizawa et al. (2016) et al. investigated the effect of different isoflurane concentrations (1.5, 2.0, 2.5, 3.0%) on mean arterial blood pressure (MAP) and BOLD signal in somatosensory cortex. While MAP was significantly lower at all higher concentrations than 1.5% isoflurane, BOLD signal was significantly higher at 2.0 and 2.5% isoflurane and decreased again at 3.0%, resulting in an inversed U-shape. Arterial blood pressure and basal BOLD signal were thus not correlated in this study.

Taken together, results are contradictory as to whether blood pressure decreases are correlated with BOLD signal changes: two studies each did or did not find correlations. Additionally, those two studies that did observe some positive correlations reported concurrent negative correlations.

3.3.1.1.7 Hypertension or increase in arterial blood pressure

In contrast to arterial blood pressure decreases, arterial blood pressure increases caused by pharmacological agents consistently caused BOLD signal increases, at least in some voxels, in five studies.

Most studies administered noradrenaline as a bolus or short infusion of maximum 1 min (Tuor et al., 2002; Wang et al., 2006; Qiao et al., 2007; Tuor et al., 2007). Voxels correlated to the arterial blood pressure time course or classified as “activated” by the noradrenaline stimulus (based on a cluster analysis) were identified in the sensorimotor cortex as well as in other brain regions. Tuor et al. (2002) noted that correlated voxels were more localised when arterial blood pressure changes were below 60 mmHg than when they exceeded 60 mmHg. The number of correlated/activated voxels typically significantly increased when a certain threshold of arterial blood pressure change, varying from 10 to 60 mmHg, depending on the region, was crossed (Wang et al., 2006; Tuor et al., 2007). In sensorimotor cortex, Wang et al. (2006) found a linear correlation between arterial blood pressure increase and the number of correlated voxels. BOLD signal increases within the correlated/activated voxels were reported to significantly correlate with the arterial blood pressure time course (Tuor et al., 2002) or to reach statistical significance (compared to baseline) at thresholds of 30 to 60 mmHg, depending on the region and condition (Qiao et al., 2007; Tuor et al., 2007). Notably, Tuor et al. (2007) investigated a stroke model 1 week after transient middle cerebral artery occlusion and observed that stroke animals showed significantly higher voxel number and signal intensity increases in the infarct and peri-infarct region for 31-45 mmHg arterial blood pressure increase than sham operated animals in the corresponding hemisphere. However, the increase in signal intensity and voxel numbers in the

contralateral, non-affected hemisphere, was significantly lower in stroke than sham animals.

Finally, one study characterised the BOLD activation pattern of an only peripherally acting cocaine derivate (cocaine methiodide) which, like cocaine, increased MAP and heart rate. Positive as well as negative BOLD signal responses were observed in some “scattered” voxels (Luo et al., 2003).

Taken together, voxels correlated to arterial blood pressure time courses and showing positive signal changes upon arterial blood pressure increases were consistently found, whereas concurrent negative signal changes were only sporadically mentioned. Typically, the number of correlated voxels and/or the signal intensity of correlated voxels were higher with higher arterial blood pressure increases. Arterial blood pressure increases of at least 30 mmHg were a common threshold for those changes to reach statistical significance.

3.3.1.1.8 Single reports

Vanhoutte et al. (2006) report a decrease of whole brain BOLD signal intensity when body temperature was increased over 38° C.

Another study observed an increase in signal intensity (ROI brain parenchyma) during normovolemic haemodilution, i.e. anaemia (Lin et al., 1998a). Signal intensity significantly decreased once the procedure was completed but did not reach baseline values during the observation period. Significantly stronger reductions of R2* (translating into stronger signal intensity increase) were found when the double amount of blood was replaced (6 instead of 3 ml).

Those reports suggest that body temperature as well as haematocrit may influence BOLD signal intensity, however, further studies would be required to characterise the effects.

3.3.1.1.9 Summary

Most studies found a modulatory effect of inspiratory gas concentrations, apnoea, blood pressure variations, body temperature and haematocrit on baseline BOLD signal. Hypoxia and apnoea under room air or room air plus CO_2 consistently decreased the BOLD signal, whereas hypercapnia, apnoea under 100% O_2 and increases in blood pressure consistently increased the BOLD signal. Hyperoxia and arterial blood pressure decreases were inconsistently reported to result in BOLD signal in- and decreases, respectively.

3.3.1.2 Effects of changes in physiological parameters on BOLD signal responses to peripheral stimulation

Fifteen references investigating the effect of physiological parameters on activations in response to peripheral stimulation were found. Thirteen of those applied electrical stimulation to the paws or in one case the cheeks, and one each used chemical somatosensory and mechanical visceral stimulation.

Eleven studies, all using electrical stimulation, deliberately modulated physiological parameters. The remaining four studies analysed the influence of naturally occurring physiological changes on BOLD responses.

3.3.1.2.1 Electrical stimulation – changes in inspiratory gas compositions

Modulations of inspiratory gas compositions had variable effects on the response to stimulation. Two studies report effects of hypoxia on response to forepaw stimulation in a way that the results cannot be directly compared (Sicard and Duong, 2005; Huang et al., 2013). On one hand Huang et al. (2013) report significantly higher BOLD signal intensity responses under 15 than under 21% O_2. On the other hand, Sicard and Duong (2005) found significantly smaller absolute BOLD signal increases (BOLD signal normalised to baseline signal under room air) under 9 than 21% O_2 and similar absolute signal increases under 12 and 21% O_2. However, relative signal increases (i.e. relative to respective baseline signal) “varied” and were described as “markedly smaller” under 9% O_2, however, the directions of these variations and whether any differences were significant is not reported.

Conflicting results were also found regarding hyperoxia. Sicard and Duong (2005) report “similar” absolute signal increases during forepaw stimulation under 21 and 100% O_2. In contrast, significantly higher numbers of activated voxels and significantly higher signal increase under 100% O_2 than under the baseline gas mixture (O_2 enriched air, 47% O_2) were found by Nasrallah et al. (2015), and arterial partial pressure of O_2 (p_aO_2) and BOLD signal change was positively correlated in both groups with similar p_aCO_2 (Nasrallah et al., 2015).

Hypercapnia, however, appears to have more consistent effects on response to forepaw stimulation. Nasrallah et al. (2015) report a significantly lower increase in signal intensity under 1 and 5% CO_2 admixture and a significantly reduced number of activated pixels under 5% CO_2 than under the baseline gas mixture (O_2 enriched air, 47% O_2). Significantly lower signal intensity and number of activated pixels were also observed under combined hyperoxia and hypercapnia (95% O_2 plus 5% CO_2). A negative correlation between pCO_2 and BOLD signal change was however only found for groups with similar p_aO_2 in this study (Nasrallah et al., 2015). Although Sicard and Duong (2005) did not find a significant difference between 5% CO_2 and room air, absolute signal changes were significantly smaller under 10% CO_2. Taken together, the two studies agree that hypercapnia reduces response to electrical forepaw stimulation.

The effect of hypercapnia as well as combined hypercapnia/hyperoxia on response to electrical forepaw and cheek stimulation was transient; no significant differences in response to stimulation were observed once p_aCO_2 returned to baseline levels after the respective gas challenges (Bock et al., 1998; Dutka et al., 2002).

Finally, one reference observed significantly higher numbers of activated pixels and signal increase in somatosensory cortex during forepaw stimulation when animals were hypocapnic (Hsu et al., 1998).

In summary, inconsistent results were often reported for the effects of specific inspiratory gas conditions on response to electrical somatosensory stimulation, and only for hypercapnia reduced responses were consistently reported.

3.3.1.2.1 Electrical stimulation – changes in arterial blood pressure

A second cluster of references investigated the effect of arterial blood pressure modulations on responses to electrical stimulation. Increases of arterial blood pressure by noradrenaline administration consistently increased the positive signal change and/or the number of voxels correlated to the forepaw stimulation time course (Wang et al., 2006; Qiao et al., 2007; Tuor et al., 2007). The threshold of blood pressure at which significance was reached varied between studies, regions and outcome measures, but was commonly in the range of 30-45 mmHg. Voxels correlated to the stimulation time course were also detected in other regions than somatosensory cortex. Notably, significant “activation” was even detected in stroke model animals when arterial blood pressure increases exceeded 15 mmHg, in which the somatosensory cortex was within the infarct region and did not display any response to stimulation under normotensive conditions (Tuor et al., 2007).

Decreases of arterial blood pressure however either enhanced (Herman et al., 2007), decreased (Hempel et al., 1999) or did not significantly change the response to stimulation (Wang et al., 2006). Low arterial blood pressures were induced in those studies via blood withdrawal, negative lower body pressure or trimetaphan camsilate, respectively.

Taken together, increases in blood pressure can be expected to increase response to forepaw stimulation, but reported results for blood pressure decreases are inconclusive.

3.3.1.2.2 Electrical stimulation – naturally occurring changes in physiological parameters

fMRI signal evoked by forepaw stimulation did not depend on the five investigated parameters p_aO_2, p_aCO_2, pH, MAP and heart rate in one study (Sumiyoshi et al., 2012).

Another study measured pCO_2 over up to 4 h and found that response to forepaw stimulation could be detected only if transcutaneous pCO_2 was less than 20% higher than baseline in spontaneously breathing animals under medetomidine or medetomidine/ketamine, or if arterial pCO_2 (measured with blood gas analysis) was below 35 mmHg (Ramos-Cabrer et al., 2005). The observation that CO_2 levels above a critical threshold reduce responses to forepaw stimulation is consistent with reports of experimentally induced hypercapnia (Sicard and Duong, 2005; Nasrallah et al., 2015).

3.3.1.2.3 Chemical somatosensory stimulation – naturally occurring changes in arterial blood pressure

A significant linear correlation between arterial blood pressure increases and BOLD signal intensity increases was observed in the first 4 minutes after formalin injection into the paw (Tuor et al., 2002). This observation is consistent with reports from noradrenaline- induced arterial blood pressure increases.

3.3.1.2.4 Mechanical visceral stimulation – naturally occurring changes in arterial blood pressure

Min et al. (2011) investigated arterial blood pressure and BOLD signal changes in response to inflation of a gastric balloon and found that arterial blood pressure changes were significantly associated with BOLD signal changes in several of the regions for which a significant number of activated voxels was detected. A quadratic model described the relationship between arterial blood pressure and BOLD signal changes most accurately (Min et al., 2011). This observation is consistent with reports from noradrenaline- induced arterial blood pressure increases.

3.3.1.2.5 Summary

In summary, hypercapnia and increases in arterial blood pressure were both in interventional and observational studies and across different stimulation modalities consistently reported to decrease and increase, respectively, the BOLD response to peripheral stimulation. Concerning arterial blood pressure decreases, the available evidence was inconclusive.

3.3.1.3 Effects of changes in physiological parameters on BOLD signal responses to central stimulation

Four references investigated the influence of arterial blood pressure changes or ventilation modes on BOLD signals detected in phMRI.

One study analysed the contribution of the change of physiological parameters on fMRI activation by investigating the time course after cocaine administration (Schmidt et al., 2006). As the fMRI signal changes persisted after heart rate, arterial blood pressure and respiratory rate returned to baseline, the authors concluded that the influence of physiologic parameter changes was negligible in this case.

A similar study compared BOLD responses to centrally acting cocaine with those to peripherally acting cocaine methiodide, which both significantly and dose-dependently increased MAP (Luo et al., 2003). While cocaine methiodide produced activations in some “scattered” voxels (see chapter 3.3.1.1.7), which were not dose-dependent, responses to cocaine (definition: (percent of activated voxels per ROI) x (AUC of signal intensity change in activated voxels)) were significantly larger and dose-dependent for positive as well as negative signal changes. Based on these observations, the authors concluded that MAP changes have not significantly contributed to the response to cocaine.

On the other hand, Kalisch et al. (2005) used phenylephrine CRI during phMRI to counteract apomorphine-induced hypotension in animals with unilateral 6-OHDA lesions of the nigrostriatal dopaminergic tract. Averaged over the 55 min imaging period, arterial blood pressure did not significantly decrease when phenylephrine was administered, as opposed to the control group without phenylephrine. Significant positive signal changes were observed with phenylephrine, but significant negative signal changes without phenylephrine. Arterial blood pressure and BOLD signal time course were significantly correlated in the control, but not the phenylephrine group. Interhemispheric differences in response to apomorphine were detected in both groups. Supported by the additional finding of significant negative signal change

without, but no significant change with phenylephrine in the control region of visual cortex, the authors conclude that arterial blood pressure decreases caused by apomorphine introduced negative signal changes which masked activations in CPu (Kalisch et al., 2005).

Xu et al. (2000) compared spontaneous and controlled ventilation for phMRI of heroin and found a significant difference: while BOLD signal globally decreased in spontaneously breathing animals, positive signal changes in specific brain regions were found in ventilated animals. The authors interpret the global signal reduction in spontaneously breathing animals as a consequence of hypoxaemia detected in arterial blood gas analysis in this (p_aO_2 significantly lower than at baseline), but not the ventilated group (Xu et al., 2000).

Taken together, arterial blood pressure changes in both directions were consistently reported to modulate BOLD responses in phMRI, however, the magnitude of effect and accordingly the authors' judgement of the relevance of these changes varied. The single report of BOLD signal decrease in supposedly hypoventilating animals is in line with reports on the effect of apnoea on baseline BOLD signal.

3.3.1.4 Effects of changes in physiological parameters on resting state BOLD measurements

One study each analysed the effect of different inspiratory gas compositions (Nasrallah et al., 2015), blood withdrawal to decrease arterial blood pressure (Kannurpatti et al., 2008), and naturally occurring fluctuations in several physiological parameters (Kalthoff et al., 2011) on rsfMRI measurements.

Nasrallah et al. (2015) report spatially more extended correlation maps for a seed in the frontlimb area of the primary somatosensory cortex (S1FL) under 100% O_2 than under baseline condition, whereas 1-5% CO_2 admixture to the baseline gas mixture (O_2 enriched air, 47% O_2) produced similar results as the baseline gas mixture. The combination of 95% O_2 and 5% CO_2 (carbogen) however reduced the spatial extent of correlation maps. Correlation strength between bilateral S1FL was significantly higher under 100% O_2 and 5% CO_2 than under the baseline condition. Depending on how the fluctuation of signal amplitude was assessed, a significant increase was observed only under 100% O_2 or also under 5% CO_2. Frequency distribution of the interhemispheric correlation was similar under baseline and 100% O_2, but the fraction of correlations in the 0.04-0.07 Hz was significantly higher in all hypercapnic conditions compared to baseline conditions. When arterial blood gas measurements were analysed instead of inspiratory gas composition, bilateral S1FL connectivity strength was not correlated with p_aCO_2. For p_aO_2, significant correlations were only found for subgroups of conditions: a negative correlation when 100% O_2 was excluded and a positive correlation when carbogen was excluded. Amplitude fluctuations were positively correlated with p_aO_2, but not p_aCO_2.

Kannurpatti et al. (2008) observed that after blood withdrawal, BOLD signal fluctuation amplitudes were enhanced and accordingly the magnitude of the power spectrum was also enhanced. The power of frequencies around 0.02, 0.03, 0.07, 0.10 and 0.125 Hz was significantly higher in the hypovolemic state. As a consequence of enhanced signal fluctuations, more voxels showed significant correlation with seed voxels placed in sensorimotor cortex, hippocampus or

thalamus, so that correlation maps appeared spatially more extended in the hypovolemic state.

In a complex linear regression, Kalthoff et al. (2011) found that in spontaneously breathing animals respiratory regressors (respiratory waveform and its derivative) explained 5% of variance after previous motion correction; when cardiac regressors (cardiac waves and its derivative) were afterwards added, variance decreased again by 1%. Respiratory rate, heart rate, SpO_2 and body temperature however were only rarely correlated to the BOLD signal and therefore not considered as regressors. In the authors' judgment, fc maps and matrices were more specific after applying their correction algorithm.

Taken together, those three reports suggest that inspiratory gas concentrations as well as arterial blood pressure decreases and respiration related physiologic noise (primarily motion) affect rsfMRI measurements, however, evidence for each parameter is only based on a single report.

3.3.1.5 Conclusion

Across experimental paradigms, results for each parameter were more consistent when the effect on baseline BOLD signal was investigated than when the effect on response to peripheral stimulation was investigated. The number of studies was too low to assess consistency of the results for central stimulation and resting state paradigms. Findings from all paradigms will be integrated per parameter in the following section.

While variable levels of hypoxia unequivocally decreased baseline BOLD signal intensity, effects on electrical paw stimulation depended on the studies and levels of hypoxia. No data was available for the effect of hypoxia on central stimulation and rsfMRI.

For hyperoxia, increased baseline BOLD signal was inconsistently reported, and results were contradictory regarding effect on response to peripheral stimulation. In a single resting state study, fc maps, interhemispheric connectivity strength and fluctuation of the signal amplitude were larger or higher under hypoxia. No data was available for central stimulation paradigms.

Hypercapnia consistently increased baseline BOLD signal and decreased response to peripheral stimulation. In a single resting state study, fc maps were not affected by hypercapnia, but interhemispheric connectivity strength increased, and the frequency distribution of the interhemispheric correlation shifted.

Combined hyperoxia and hypercapnia was reported by a single study to increase baseline BOLD signal, decrease the spatial extent of fc maps and shift the frequency distribution of interhemispheric correlations. No data was available for its effect on peripheral and central stimulation paradigms.

Apnoea under room air and supposed hypoventilation in spontaneously breathing animals reduced the baseline BOLD signal and response to central stimulation, respectively, in one study each.

Interestingly, findings for increases in blood pressure were consistent across experimental paradigms, whereas those for decreases were controversial within each paradigm. Increases in blood pressure increased both the baseline BOLD signal and

responses to different modalities of peripheral stimulation, including chemical somatosensory and visceral stimulation. Only two observational studies addressed phMRI and interpreted eventual contributions from arterial blood pressure increases as negligible. On the other hand, reported effects of blood pressure decreases varied from no effect on to decreased baseline BOLD signal and from reduced over unchanged to enhanced responses to peripheral stimulation. One study each found a positive correlation of arterial blood pressure time course and BOLD signal time course in an phMRI where the test substance induced hypotension, and increased signal amplitude fluctuation and spatially more extended fc maps under lower blood pressure in rsfMRI.

In summary, the majority of studies found that modulations of physiological parameters, notably by changing inspiratory gas concentrations or manipulating arterial blood pressure, affected BOLD signal measurements across experimental paradigms. There was a clear trend that interventional studies more commonly identified an effect than observational studies (no difference in 3 out of 39 interventional versus 3 out of 6 observational studies).

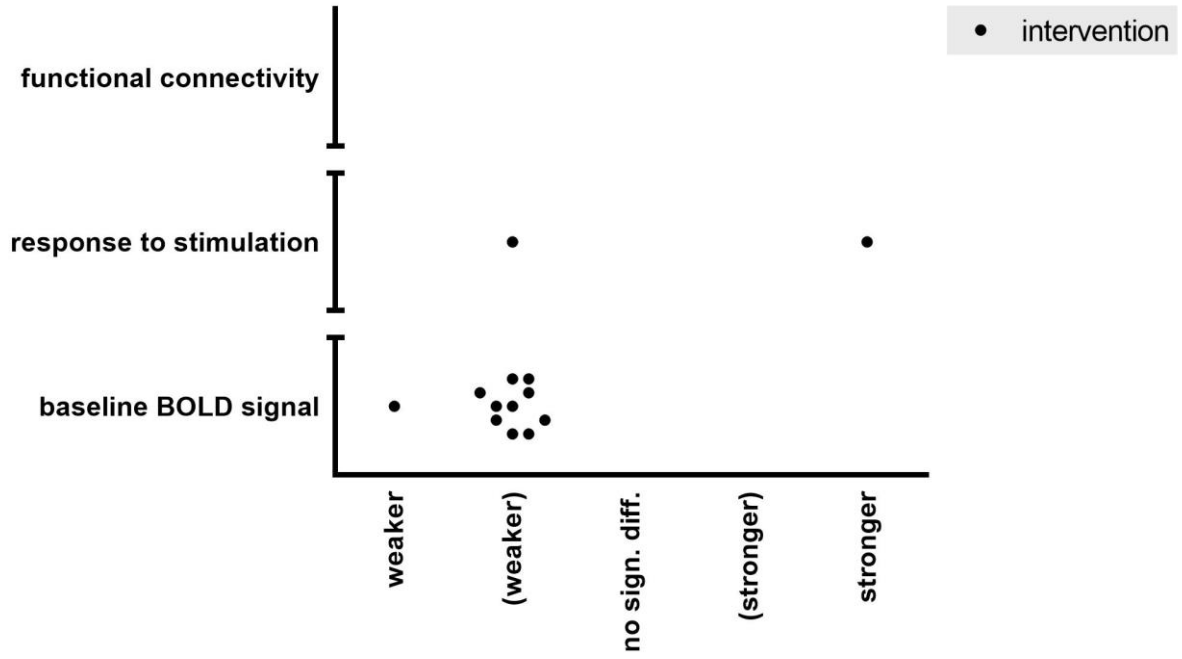

Figure 7. Effects of experimentally induced hypoxia, compared to respective baseline condition, on baseline BOLD signal and responses to stimulation in rats (no data available for effects on fc). stronger = higher BOLD signal or lower R2*, or higher signal intensity and/or spatial extent of activated area upon stimulation under hypoxia; (stronger) = BOLD signal or response to stimulation stronger in some, but

not all aspects under hypoxia; no sign. diff. = no significant difference between respective baseline condition and hypoxia. “weaker” and “(weaker)” analogous to “stronger” and “(stronger)”. One data point per experimental paradigm per dataset and anaesthetic. If no statement on the significance of reported changes was available, “(stronger)” or “(weaker)” were selected.

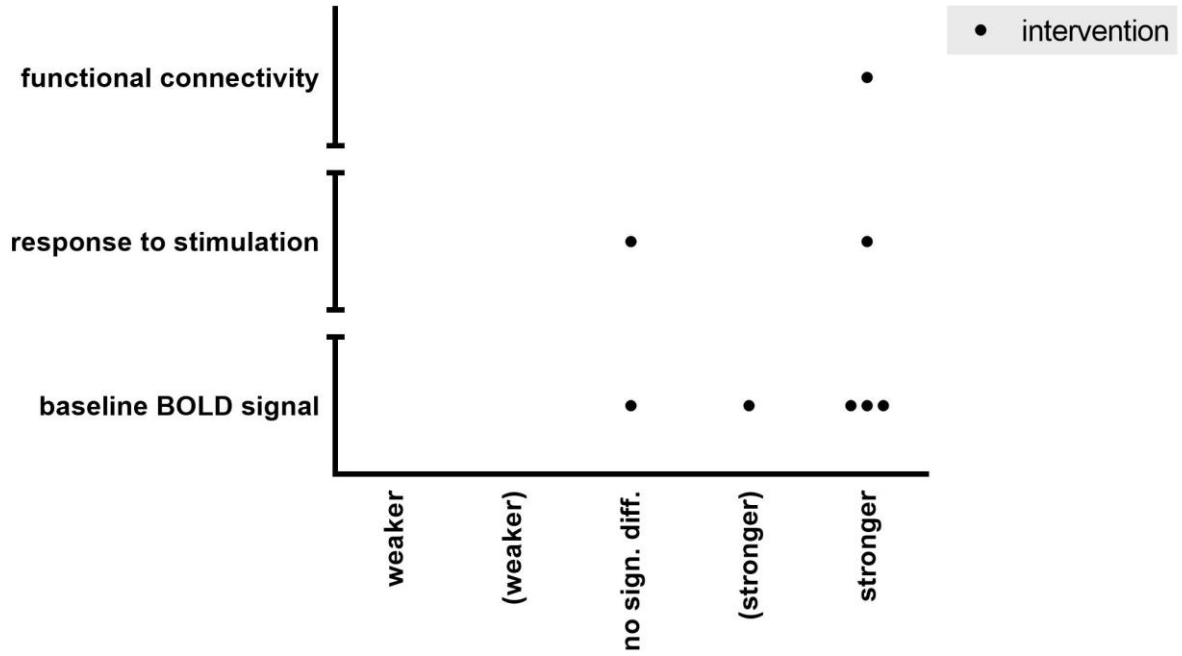

Figure 8. Effects of experimentally induced hypoxia, compared to respective baseline condition, on baseline BOLD signal, responses to stimulation and fc in rats. stronger = higher BOLD signal or lower R2*, higher fc strength and/or spatial extent of connectivities or higher signal intensity and/or spatial extent of activated area upon stimulation under hyperoxia; (stronger) = BOLD signal or response to stimulation stronger in some, but not all aspects under hyperoxia; no sign. diff. = no significant difference between respective baseline condition and hyperoxia. “weaker” and “(weaker)” analogous to “stronger” and “(stronger)”. One data point per experimental paradigm per dataset and anaesthetic. If no statement on the significance of reported changes was available, “(stronger)” or “(weaker)” were selected.

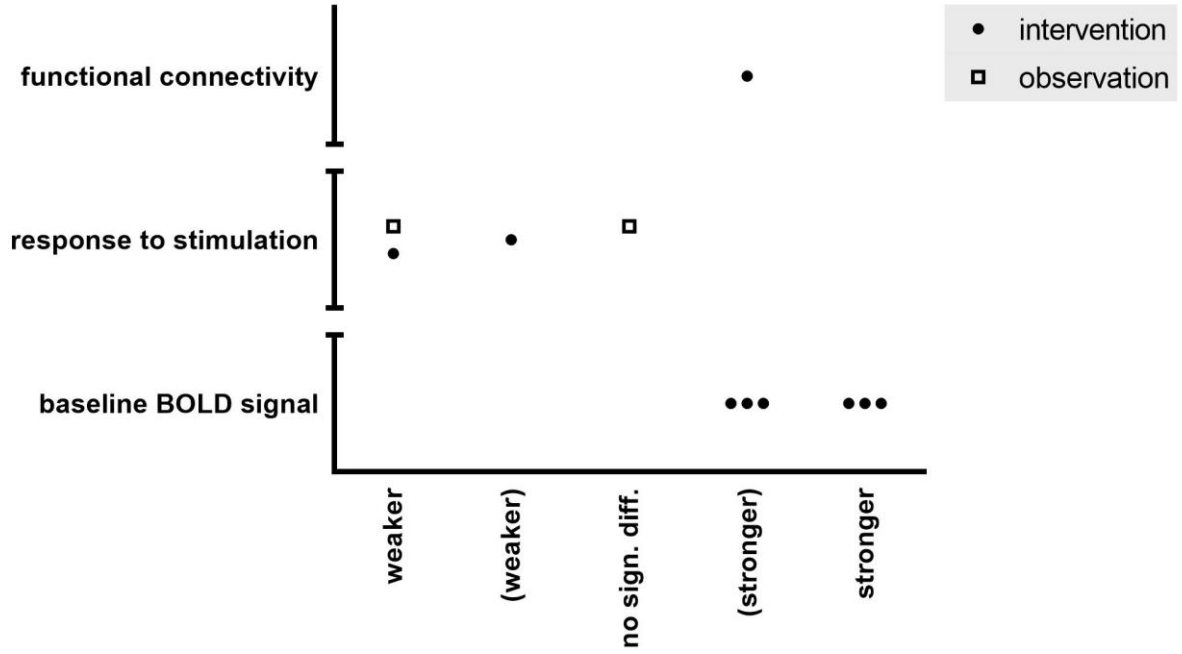

Figure 9. Effects of experimentally induced hypercapnia (intervention) or of naturally occurring increases of p_aCO_2 (observation), compared to respective baseline condition, on baseline BOLD signal, responses to stimulation and fc in rats. stronger = higher baseline BOLD signal, higher fc strength and/or spatial extent of connectivities or higher signal intensity upon stimulation and/or spatial extent of activated area under hypercapnia/higher p_aCO_2; (stronger) = baseline signal, fc or response to stimulation stronger in some, but not all aspects under hypercapnia/higher p_aCO_2; no sign. diff. = no significant difference between awake and anaesthetised. “weaker” and “(weaker)” analogous to “stronger” and “(stronger)”. One data point per experimental paradigm per dataset and anaesthetic.

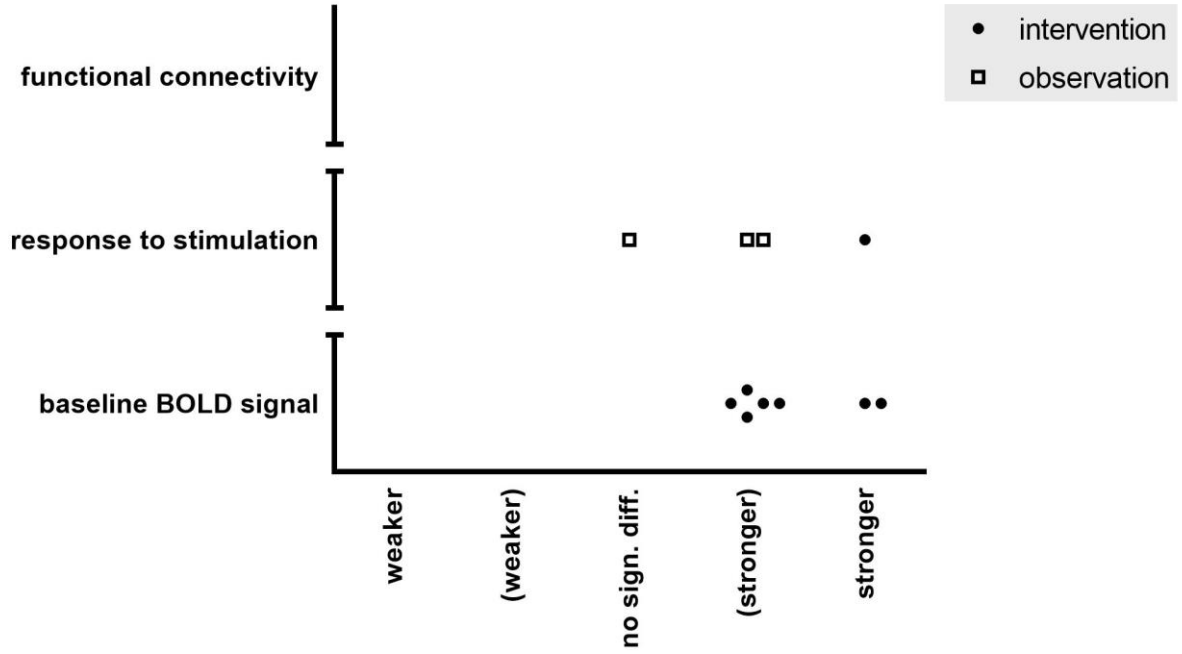

Figure 10. Effects of experimentally induced (= intervention) or naturally occurring (= observation) blood pressure increases on baseline BOLD signal and responses to stimulation in rats (no data available for effects on fc). stronger = higher baseline BOLD signal or higher signal intensity upon stimulation and/or spatial extent of activated area when blood pressure increased; (stronger) = baseline signal or response to stimulation stronger in some, but not all aspects when blood pressure increased; no sign. diff. = no significant difference associated with blood pressure increases. “weaker” and “(weaker)” analogous to “stronger” and “(stronger)”. One data point per experimental paradigm per dataset and anaesthetic. If positive and negative correlations of the BOLD signal with blood pressure were observed, “(stronger)” was selected in one case to indicate that a relevant difference was observed and “no significant difference” in another case where changes were limited to a few voxels and considered irrelevant by the authors.

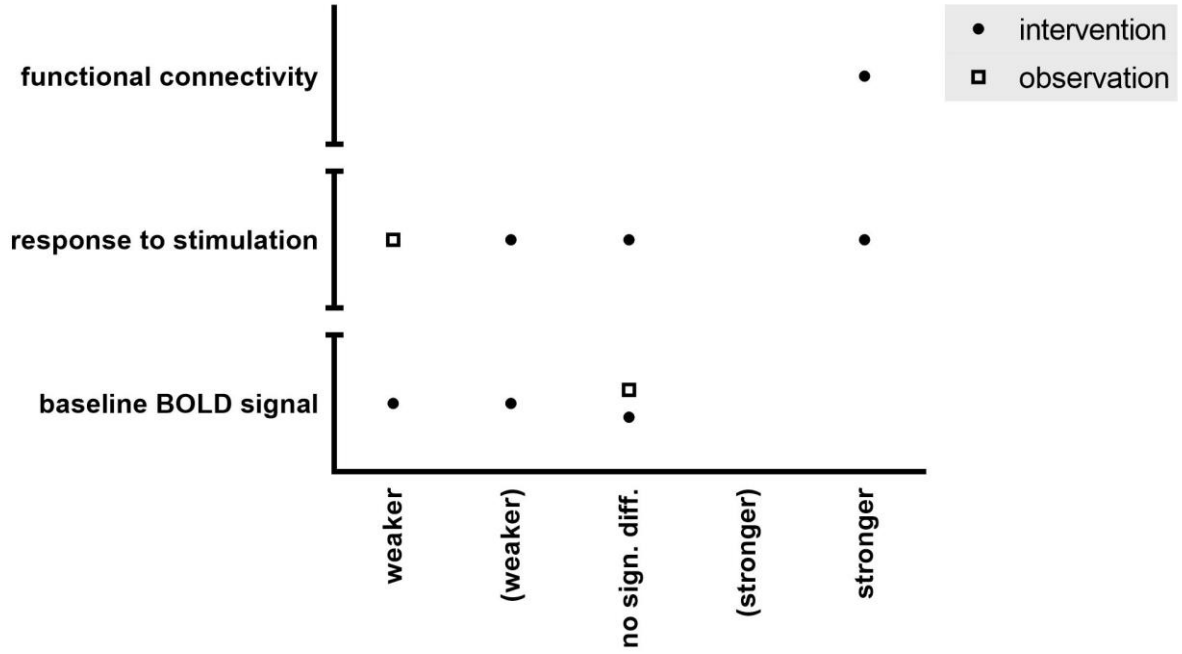

Figure 11. Effects of experimentally induced (= intervention) or naturally occurring (= observation) blood pressure decreases on baseline BOLD signal, responses to stimulation and fc in rats. stronger = higher baseline BOLD signal, higher fc strength and/or spatial extent of connectivities or higher signal intensity and/or spatial extent of activated area upon stimulation when blood pressure decreased; (stronger) = baseline signal, fc or response to stimulation stronger in some, but not all aspects when blood pressure decreased; no sign. diff. = no significant difference associated with blood pressure decreases. “weaker” and “(weaker)” analogous to “stronger” and “(stronger)”. One data point per experimental paradigm per dataset and anaesthetic. In one case positive and negative correlations of the BOLD signal with blood pressure were observed and “(weaker)” was selected to indicate that a relevant difference was observed.

3.3.2 Mice

Four references addressed the effect of physiological parameter variations on baseline BOLD signal or response to electrical hind paw stimulation in mice. One study modulated inspiratory gas concentrations; three studies analysed naturally occurring changes in physiological parameters.

Under 100% O_2, R2* in the striatum was significantly lower than under air plus 10% CO_2, and under air plus 10% CO_2, R2* in the striatum was significantly lower than under room air, which translates to increasing baseline signal from air to air plus CO_2 to 100% O_2 (Sedlacik et al., 2015).

Schroeter et al. (2014) observed that unilateral electrical hindpaw stimulation lead to widespread, bilateral activation including regions not involved in somatosensory processing under all four anaesthetic agents used in that study. As heart rate, pulse distension and SpO_2 changed in response to stimulation, the authors concluded that an unspecific arousal response accounts for these signal increases. Except for SpO_2, which was used together with CBV to calculate relative O_2 delivery, which linearly correlated with the BOLD signal change, the relation between physiological parameters or their change during stimulation and the BOLD signal or its changes was however not quantitatively analysed. In a follow-up study (Schlegel et al., 2015), randomised single pulse stimuli were used instead of a block stimulation paradigm. With this paradigm, no changes in physiological parameters were observed, activation maps were spatially less extended, BOLD responses in visual cortex (serving as control region) were “strongly reduced”, and BOLD signal amplitudes in ipsilateral hindlimb area of the primary somatosensory cortex (S1HL) reached only approximately 60-80% of contralateral cortex responses, as opposed to equivalent responses to block stimulation. Again, the relation between physiological parameters and the BOLD signal was not quantitatively analysed. Another study by the same group showed that bilateral responses to unilateral hindpaw stimulation also occurred in acallosal mice (Schroeter et al., 2017) which the authors interpreted as a strong argument for the cardiovascular origin of the ipsilateral response.

Taken together, single reports of experimentally altered inspired gas concentrations (hypercapnia, hyperoxia and hypoxia) suggest that the baseline BOLD signal in mice is modulated. Qualitative observational studies further suggest that cardiovascular responses elicited by somatosensory stimulation contribute to the measured BOLD signal changes. Overall, however, only limited evidence is available for influences of physiological parameter alterations on BOLD fMRI measurements in mice.

3.4 BOLD responses and functional connectivity in awake animals and under different anaesthetic conditions

3.4.1 Rats

3.4.1.1 Effects of different states of anaesthesia on BOLD responses to experimental changes in physiological parameters

Five articles specifically investigated whether and how BOLD responses to manipulation of physiological parameters differed under different states of anaesthesia.

Duong (2007) studied three levels of hypoxia (9, 12, and 17% inspiratory O_2 concentration) in awake and isoflurane (2%)-anaesthetised, spontaneously breathing animals. A significant difference in responses was only observed when pa O_2 was below 50 mmHg: the BOLD signal decrease was stronger under isoflurane.

Brevard et al. (2003) and Sicard et al. (2003) both compared responses to hypercapnia in awake and isoflurane (2%)-anaesthetised, spontaneously breathing animals. Both observed a significantly higher signal increase in awake animals when

exposed to 5 or 10% CO_2 in a normoxic gas mixture. Sicard et al. (2003) covered the entire brain and Brevard et al. (2003) a cortical and a subcortical ROI. In the cortical ROI, responses to CO_2 were dose-dependent in both states of consciousness, whereas in subcortical ROI the signal change was only in awake animals dose-dependent. Brevard et al. (2003) additionally noted significantly shorter rise times of the signal and Sicard et al. (2003) higher baseline MRI fluctuations in the awake condition.

Kannurpatti and Biswal (2004) compared responses to apnoea starting from three different gas mixtures under urethane (1.2 g/kg intraperitoneal (ip)) and pentobarbital (60 mg/kg ip). Neither when starting from 100% O_2 nor from 2 or 5% CO_2 (probably added to room air, but not specified) any differences in the respective BOLD responses (amplitude, time to onset, time to peak) were observed between anaesthetics. Only when starting from room air, the time to maximal signal decrease was significantly longer under pentobarbital than under urethane.

Kalisch et al. (2001) induced blood pressure decreases of variable degree by repeatedly withdrawing and re-infusing blood from isoflurane-, halothane- or propofol-anaesthetised animals. In all three groups, significant correlations between blood pressure time courses and whole brain slice BOLD time courses were found. Due to small group sizes (3 animals per condition), the authors however explicitly refrained from characterising anaesthetic specific profiles of those correlations.

Taken together, mainly single reports suggest that responses to hypoxia, hypercapnia, apnoea and decreases of blood pressure are qualitatively similar under different anaesthetics. Presence and direction of effects were consistent across groups. However, significant quantitative differences were found in responses to apnoea under room air, and alterations in inspiratory gas concentrations under urethane versus pentobarbital and isoflurane versus awake, respectively. Interestingly, the direction of the difference was opposite under hypoxia and hypercapnia: while upon hypercapnic stimulation stronger responses were observed in awake animals, hypoxic stimulation (if exceeding a certain level of severity) produced stronger responses in anaesthetised animals.

3.4.1.2 Effects of different states of anaesthesia on resting state BOLD measurements

34 articles investigated effects of different anaesthetic protocols and awake imaging on rsfMRI. Most information is available for the comparison of fc in the awake state versus under isoflurane, under varying levels of isoflurane, propofol or medetomidine and for the comparison of isoflurane with (dex)medetomidine or a combination of isoflurane and (dex)medetomidine. Isoflurane and propofol were only compared in one conference abstract with very limited detail of results and no comparisons of propofol with other anaesthetics were found.

Some reports of ketamine, either administered on top of medetomidine, in combination with medetomidine or in combination with the α_2-agonist xylazine (in the last case compared to isoflurane) were also found.

Only single reports are available for fc under α-chloralose, urethane and thiobutabarbital, especially in comparison to other anaesthetics.

3.4.1.2.1 Isoflurane versus awake

Seven articles based on three datasets compared fc in the awake state with fc under various concentrations of isoflurane. All articles found a reduction of overall fc strength (absolute and/or relative), fc between specific areas, or the temporal occurrence of specific fc patterns under isoflurane.

One study reports “mainly […] localised connections” of the somatosensory cortices under isoflurane, while in awake animals, a seed placed in S1HL demonstrated fc with its contralateral counterpart, secondary somatosensory cortex (S2) and insular cortex (Chang et al., 2016). Similar findings were observed when random stimulation (air puffs) was delivered to a paw to simulate chronic pain conditions. Whole brain fc, assessed from pairwise correlation coefficients of 264 anatomically defined ROI, was stronger in awake animals (Chang et al., 2016).

Another study found in thalamocortical and frontoparietal networks under isoflurane (0.5-3%) more commonly reduced than enhanced connectivity within the networks and a spatially homogenous reduction of averaged normalised fc (i.e. averaged value of its connections to all other voxels, normalised to the awake value, for each voxel) under isoflurane of 1.5% or higher (LORR at 1.5% under scanner conditions in this study due to delivery via nosecone) (Hamilton et al., 2017). For all doses of isoflurane, averaged normalised fc change was negative in all voxels compared to the awake state. Absolute fc changes (between 134 unilateral ROI as well as between each pair of voxels in the brain) were negatively correlated with the fc strength in the awake state. Entropy of the BOLD signal time course, a measure for the randomness of the signal, was significantly higher under isoflurane, while mutual information was significantly lower than in the awake state. Re-analysis of the same dataset revealed a significant decrease of fc strength between ROI pairs in static fc analysis, but similar spatial patterns between conditions, manifesting as high spatial correlation coefficients between RSFC matrices across conditions (Ma et al., 2017). Clustering of all pairwise correlation matrices obtained from sliding windows from all levels of isoflurane, including awake, into five recurring connectivity patterns revealed a pattern that most commonly occurred in the awake state and almost disappeared for isoflurane concentrations of 3% and higher. Nevertheless, all patterns occurred in all conditions. Interestingly, the first pattern was the least similar to the static connectivity patterns, whereas pattern number 5, the most similar to the static connectivity patterns, dominated at the highest isoflurane level.

Three studies (Liang et al., 2013; Liang et al., 2015a; Smith et al., 2017) all re-analysed (at least some) data from Liang et al. (2012a). The original article focuses on anti-correlation between the infralimbic cortex and the amygdala, which was absent in anaesthetised compared to awake rats (Liang et al., 2012a).

Liang et al. (2013) report reduced thalamocortical connectivity “in all thalamocortical networks” (seed-based fc analysis for eight bilateral thalamic seeds) under isoflurane.

Smith et al. (2017) report reduced fc of a seed placed in the claustrum with medial prefrontal cortex and mediodorsal thalamus, but not with cingulate and agranular cortex as well as with the contralateral claustrum. The same study also reports a decreased fc between a seed in prelimbic cortex (part of medial prefrontal cortex) and the claustrum as well as the mediodorsal thalamus.

Liang et al. (2015a) investigated both static and dynamic fc in infralimbic and primary somatosensory networks. When seeds were placed bilaterally in infralimbic cortex,

both conventional correlation analysis and averaging of the co-activations observed in selected single frames (15% highest BOLD signal in seed) demonstrated “consistently weaker” and spatially more confined connectivity under anaesthesia. For a seed placed unilaterally in barrel field of the primary somatosensory cortex (S1BF), co-activation and correlation patterns were judged “similar” under both states of consciousness. Dynamic fc was analysed by clustering patterns from individual frames in the awake state into three co-activation patterns (CAP). For the infralimbic seed, the first CAP (CAP1) encompassed limbic and some prefrontal regions, the second CAP (CAP2) motor and somatosensory cortices, and the third CAP (CAP3) the hippocampus, with the first CAP observed most and the last least frequently. Under anaesthesia, the connectivity of the seed with limbic regions was reduced in CAP1, the strength of co-activation generally reduced in CAP2 (while the spatial pattern persisted) and the co-activation in CAP3 “virtually disappeared”. Emergence rate of CAP1, i.e. the percentage of selected frames displaying a certain CAP, decreased and of CAP3 increased significantly. For the somatosensory seed, the three CAP observed in the awake state were also observed in the anaesthetised state (higher within-cluster similarity between states than infralimbic network), with the exception of primary somatosensory cortex (S1)-hippocampus co-activation in CAP3 which was absent. Emergence rates did not significantly differ between states of consciousness. Overall, both static and dynamic fc were more affected for a seed placed in infralimbic cortex, i.e. in networks supposed to support cognitive and emotional functions, than in sensorimotor networks, and dynamic fc was more sensitive at revealing anaesthesia-related differences than static fc.

The references presented here focused on different regions and networks, used variable methods of analysis and analysed the differences between awake and anaesthetised scans at variable level of detail. In conclusion, they consistently report reduced fc under isoflurane compared to the awake state and provide evidence that isoflurane anaesthesia affects different networks to different degrees.

3.4.1.2.2 Isoflurane concentrations

Nine articles based on eight datasets compared different concentrations of isoflurane.

Hamilton et al. (2017) report a significant increase of entropy (a measure of randomness of the BOLD signal in individual regions) with increasing isoflurane concentrations (0.5-3.0%), reaching a plateau at 1.5%, whereas mutual information (a measure for the information exchanged between two brain regions) significantly decreased with increasing isoflurane, not reaching a plateau. Absolute fc changes (between 134 unilateral ROI as well as between each pair of voxels in the brain) were negatively correlated with the fc strength in the awake state, and the correlation was significantly more negative, the higher the isoflurane concentration was. Ma et al. (2017), based on the same dataset, report monotonic decrease of static fc with increasing isoflurane concentration in spatially similar (static) connectivity patterns, and dynamic fc patterns that are in contrast most common at low (pattern 1), high (pattern 5) or intermediate (pattern 3) isoflurane concentrations.

As isoflurane concentrations were increased from 1 to 3%, Nasrallah et al. (2014a) observed a significant reduction of interhemispheric S1FL connectivity and a concurrent significant reduction of total power of the BOLD signal fluctuations.

Liu et al. (2011) also found a reduced magnitude of BOLD signal fluctuation at 2.0 versus 1.8% isoflurane and a reduced coherence strength at 2.2 versus 1.8% isoflurane. Qualitatively, bilaterally synchronous “bumps” were observed in the S1FL reference regions under 1.8 and 2.0, but not 2.2% isoflurane. Correlation coefficients of voxels correlated with the seed region were generally weaker under 2.2% than under the lower concentrations and spatially less extended (for 1.8 and 2.0%, “strong correlation over the majority of cortical regions” is reported. Weaker correlations with CPu and thalamic nuclei are reported primarily for 1.8% isoflurane).

In contrast, Pan et al. (2011) observed an increase of interhemispheric fc when isoflurane was increased from 1.0 to 1.8%.

Similarly, Liu et al. (2013b) report spatially less specific correlation maps when isoflurane was increased from 1.0 to 1.8%. This range of isoflurane concentrations complements the range studied earlier study by the same group (Liu et al., 2011). Both studies report strong correlations in “most cortical and some subcortical regions” (Liu et al., 2013b) relative to a seed in S1FL at the shared concentration of 1.8% isoflurane; at 1.0%, correlations were mainly confined to interhemispheric correlation of the seed (in S1FL or S1BF) with its counterpart. At 1.8%, but not at lower concentrations, the 2013 study also observed the “bumps” in BOLD signal time courses described in the earlier study. Interestingly, signal fluctuations were smallest at 1.5%, i.e. at the intermediate concentration of those tested in the later study (Liu et al., 2013b). Power spectra analysis revealed a higher power in all frequencies at 1.0% than at 1.5 and 1.8%, in line with the findings reported by Nasrallah et al. (2014a) for a broader range of isoflurane concentrations.

In addition to spatial, Wang et al. (2011) also investigated temporal aspects of fc. ROI were defined in bilateral S1, S2, primary motor (M1) and secondary motor (M2) cortices plus CPu and ventral posterolateral thalamic nucleus (VPL). The relative amplitude of BOLD signal fluctuations was significantly reduced at 2.9% isoflurane compared to 0.5% isoflurane in all ROI. Within-ROI connectivity (i.e. mean correlation of all voxels within the ROI with the ROI’s reference signal time course) was significantly reduced at 2.9 versus 0.5% isoflurane in all cortical ROI and CPu. Interhemispheric correlation coefficients between homotopic regions was significantly decreased for M1, M2 and CPu at 2.9 versus 0.5% isoflurane, but not for S1FL, S2 and VPL. Temporal characteristics were assessed by the Hurst exponent. While a Hurst exponent of 0.5 is equivalent to white noise, values above 0.5 indicate that the signal has a “positive autocorrelation over long time lags”. In contrast, values below 0.5 represent an anticorrelated signal. H was over 0.5 in all ROI at 0.5 and 1.0% isoflurane, but significantly lower (in relation to 0.5%) at 2.9% isoflurane in all cortical ROI and CPu, approaching 0.5. This finding was interpreted by the authors as resting state signal fluctuations being driven by spontaneous neurophysiological events at lower isoflurane concentrations, but not containing meaningful information at higher concentrations anymore (abolition of spontaneous activity).

One study did not address conventional fc measures, but instead calculated degrees of freedom (DOF) of the BOLD signal (Kundu et al., 2014). A high degree of freedom was interpreted as a higher amount of structured information within the signal and thus considered desirable. DOF were consistently reduced when isoflurane concentrations were increased from 1.0 to 1.5 and 2.0%.

So far, all references investigated healthy animals. Gill et al. (2017) report the effect of different isoflurane levels on fc in a kainic acid model of temporal lobe epilepsy.

Connectivity matrices between 33 brain ROI were generated: those ROIs which connectivities' differed significantly between kainite treated and control animals were defined as nodes, and significant correlations between nodes as (undirected) edges. At 2.0% isoflurane, the number of nodes and edges (7 and 6) was reported to be "greatly diminished" compared to 1.5% (23 and 78), although a minority of nodes were only observed at 2.0%. Differences in global network parameters as well as the regional network parameter betweenness centrality, between kainite treated and control animals, were consistent across isoflurane levels. Only for small worldness of the network, two parameters out of three (ω and γ, but not λ) differed significantly between groups at 1.5, but not 2.0% isoflurane.

In conclusion, studies consistently report reduced amplitudes of signal fluctuation under higher isoflurane concentrations, but while some studies found reduced strength of fc and spatial extension of correlated regions, others report increased, unspecific correlations for higher isoflurane concentrations.

3.4.1.2.3 Propofol doses

Four articles based on three datasets investigated fc under different levels of propofol. Tu et al. (2011) report absent thalamocortical fc under 160 mg/kg propofol ip, whereas under 80 mg/kg ip connectivity between right thalamus, ipsilateral S1 and contralateral S2 were observed. Hudetz et al. (2015) compared the repertoire of dynamic "brain states" by calculating regional homogeneity (ReHo) and coincident threshold crossing from a sliding window approach under 20 versus 40 mg/kg/h of propofol intravenously (iv). ReHo is a measure for the similarity of a voxel's time course with its 27 closest neighbours' time courses. Voxels exceeding the mean ReHo by 2 standard deviations (SD) were selected and the temporal variance of ReHo values interpreted as representing the repertoire of brain states. Coincident threshold crossing selects per image all voxels which cross a threshold defined as a multiple of each voxel's SD. The number of voxels crossing the threshold and their average BOLD signal were extracted, and the temporal variability of the average BOLD signal interpreted as representing the repertoire of brain states. Temporal variance of both measures was significantly reduced under the higher propofol dose. The number of unique brain states identified from coincident threshold crossing by clustering was reduced from 81 to 66 under the higher propofol dose. When 12 anatomically defined ROI were analysed, visual cortex, parietal cortex and CPu contributed most to the global reduction of temporal variance, while changes in subcortical structures contributed relatively little. Interestingly, temporal variance of selected coincident threshold crossing voxels as well as the relative change under the higher propofol dose increased from lateral to medial, which was interpreted as potential activity and depression of default mode network under the lower and higher dose, respectively. Cross-correlation between significant ReHo areas was not different between doses, therefore the authors conclude that temporal dynamics rather than the degree of regional connectivity are a target of anaesthesia.

Hudetz et al. (2016) re-analysed the data from Hudetz et al. (2015) and found a difference in the localization of selected voxels under the two doses ("visually evident"), but no difference in the average ReHo of selected voxels. Lempel-Ziv complexity, a proxy for "the amount of non-redundant information contained in a string", which estimates "the minimal number of character sequences […] required to

describe the string", was significantly higher under the lower propofol dose, i.e. lighter plane of anaesthesia. Finally, Liu et al. (2013a) found generally reduced whole brain and regional fc when propofol infusion rates were over 20 mg/kg/h iv (40/60/80/100 mg/kg/h incrementally), however, effects were region-dependent and multiphasic patterns commonly observed (e.g. initial reduction with increasing dose followed by partial recovery of fc strength). Overall, cortical fc was reduced at lower doses than subcortical fc and while subcortical fc increased again at doses associated with burst-suppression in EEG, this was not observed for cortical fc. A potential limitation of this study is that animals were hypoxic (SpO_2 89-91%) throughout conditions.

In conclusion, either a reduction of conventional static fc or a diminished repertoire of brain states was consistently observed with higher doses, however, decrease of regional fc was controversial: while Liu et al. (2013a) observed it, Hudetz et al. (2015) did not. A single study suggests that dose-dependent effects are non-monotonic and region-dependent.

3.4.1.2.4 Isoflurane versus propofol

Only one conference abstract briefly reports "generally higher" inter- and intrahemispheric fc in isoflurane-anaesthetised animals than in propofol-anaesthetised animals (significant effect of anaesthetics in ANOVA) (Boonzaier et al., 2017). A direct comparison of propofol with dexmedetomidine, which was also investigated in that study, is not reported. How the patterns of fc observed under propofol could be characterized in relation to other anaesthetics or the awake state remains therefore unknown.

3.4.1.2.5 Medetomidine doses

Three studies investigated different rates of medetomidine CRI and one investigated the impact of different duration of previous isoflurane exposition.

Nasrallah et al. (2012; 2014a) compared 0.1, 0.2 and 0.3 mg/kg/h medetomidine infusion rates in two separate studies. Interhemispheric correlation between bilateral S1 and S2, but not CPu, was significantly reduced at 0.2 and 0.3 mg/kg/h compared to 0.1 mg/kg/h (Nasrallah et al., 2012). The amplitude of BOLD signal fluctuations was however not significantly different across infusion rates. Frequency analysis revealed that correlations in the 0.01-0.04 Hz range, the range of strongest correlations for S1 and S2, were significantly reduced at the higher two infusion rates. Additionally, correlations in the 0.04-0.07 Hz range and for S2 also in the 0.07-0.1 Hz range were significantly reduced at higher doses. No significant difference in any of the frequency bands was observed in CPu. The second study also reports significantly reduced interhemispheric connectivity between bilateral S1FL under higher doses, without any significant impact on the amplitude of signal fluctuations and the total power (Nasrallah et al., 2014a).

Based on the observation that sedation from medetomidine-CRI is limited to a couple of hours, Pawela et al. (2009) evaluated the effect of medetomidine infusion rate changes after 120 min of constant 0.1 mg/kg/h on fc. While a secondary rate of 0.1 mg/kg/h was generally associated with a decrease of pairwise correlation coefficients in the correlation matrix of 10 ROI (including S1, S2 and CPu), an increase of

correlation coefficients was observed with 0.3 mg/kg/h as the secondary rate in individual ROI pairs (changes significant for some pairs, not for others).

As (dex)medetomidine CRI is often initiated after induction with isoflurane, Magnuson et al. (2014) investigated how the duration of previous isoflurane exposition affects fc observed under dexmedetomidine CRI (0.05 mg/kg/h subcutaneously (sc), 80 min after bolus increased to 0.15 mg/kg/h sc). 45 min after the initial dexmedetomidine bolus (0.025 mg/kg sc), connectivity between bilateral S1 was significantly higher in animals exposed to 30 min of isoflurane (short isoflurane group) than in animals exposed to 3 h of isoflurane (long isoflurane group). S1-S1 connectivity significantly increased over time in the long isoflurane group. Whole brain connectivity (i.e. correlation of each individual voxel's signal time course with whole brain signal time course) was also significantly higher in the short isoflurane group at 45 min after the bolus and additionally at 105 min after the bolus. Maximum power was significantly higher in the short isoflurane group at some (75 min, 105 min, 135 min after bolus), but not all timepoints; the same applies for low- and highband (short isoflurane > long isoflurane at 75 min, 105 min and 165 min after bolus) and broadband (significant at 135 min) frequencies. Qualitatively, the power in low band frequencies increased at 1.25 h in the short isoflurane group but reached a similar level only at 3.25 h in the long isoflurane group. Accordingly, a significant increase of power in all three bands in the second half of the observation period (3.75-5.75 h after bolus) was found in the long isoflurane group, whereas a significant increase of power was observed in the short isoflurane group only in the broad- and high-band frequencies.

In summary, Nasrallah et al. (2012) and Pawela et al. (2009) both suggest regional differences in the susceptibility of fc to medetomidine. The time frames covered by the studies are complementary, Nasrallah et al. (2012, 2014a) acquiring rs scans approximately 50 min after start of the medetomidine infusion over 10 min and Pawela et al. (2009) acquiring 10 rs scans of 3 min 40 s starting from 30 min after the rate change (150 min after start medetomidine infusion). Taken together, the presented studies suggest that while initially fc is lower under higher infusion rates, fc is not stable over prolonged periods of CRI and additionally affected by the duration of previous isoflurane exposition.

3.4.1.2.6 Isoflurane versus (dex)medetomidine

Five studies compared isoflurane with medetomidine or its active enantiomer dexmedetomidine.

An abstract briefly reports "generally higher" inter- and intrahemispheric fc in isoflurane-anaesthetised than in dexmedetomidine-sedated animals (significant effect of anaesthetics in ANOVA) (Boonzaier et al., 2017).

Kalthoff et al. (2013) used several different analytical approaches to compare fc under isoflurane (1.5%) and medetomidine (0.1 mg/kg/h sc). Seed-based fc maps under isoflurane displayed "widespread cortical or subcortical correlations", regardless of the chosen seed, whereas connectivity between bilateral homotopic regions was dominant in maps acquired under medetomidine. Global signal regression reduced the widespread, supposedly non-specific correlations, but interhemispheric connectivity remained weak compared to medetomidine. Pairwise correlation of ROI resulted in higher, although less specific interhemispheric

correlations under isoflurane, which were stronger reduced by global signal regression than those observed under medetomidine. Notably, correlation strength between regions diminished with distance between regions under medetomidine (before global signal regression), but not under isoflurane. So far, it appears that fc was more specific and robust under medetomidine than under isoflurane. In line with this, independent component analysis (ICA) revealed a higher incidence of cortical and striatal networks under medetomidine than under isoflurane. Under medetomidine, networks consistently segregated into medial, lateral and intermediate components, and striatal components were observed at a high incidence, which was both not the case under isoflurane.

Williams et al. (2010) administered animals first medetomidine (0.1 mg/kg/h), followed by 2% isoflurane. As described by Kalthoff et al. (2013), lower and more localized correlations were observed under medetomidine when correlation maps were created for seeds in S1, S2 and CPu. Average correlation values for voxels in contralateral S1 were higher under isoflurane. However, under isoflurane high correlation coefficients were observed in widespread cortical areas to the degree that fc maps for seeds in S1 and S2 did not show a clear difference. Meanwhile, high correlation coefficients in bilateral homotopic regions resulted in seed-specific maps under medetomidine. In pairwise ROI analysis, high correlations were observed in all pairs under isoflurane and for bilateral homotopic regions under medetomidine. Correlation between ipsilateral S1 and S2 and S2 and CPu were significantly lower under medetomidine than under isoflurane. Power spectra of the BOLD signal did not differ between anaesthetics.

Paasonen et al. (2016a) compared among others isoflurane and medetomidine in a combined resting state and pharmacological fMRI. Fc was stable over time (1 h) under both protocols. Correlation between averaged connectivity with other regions in baseline rs scans and responses to nicotine stimulation for each ROI (14 total) yielded high values, especially in cortical regions, under isoflurane, whereas under medetomidine, consistent correlations were found primarily in subcortical regions.

Regarding dose-dependence of the rs BOLD signal, Nasrallah et al. (2014a) found that under both isoflurane (1.0, 2.0 and 3.0%) and medetomidine (0.1, 0.2 and 0.3 mg/kg/h ip after 0.05 mg/kg ip), interhemispheric connectivity between bilateral S1FL was significantly reduced under higher doses. However, while increasing the level of isoflurane reduced the amplitude of signal fluctuations and the total power, this was not the case when medetomidine rate was increased.

Taken together, studies consistently report more localized and lower correlations under medetomidine, which are commonly interpreted as more specific than those observed under isoflurane. The single study which evaluated several doses of both drugs found for both a reduction of fc when higher doses were used. This is in line with the observations of Liu et al. (2011), Nasrallah et al. (2012) and Ma et al. (2017).

3.4.1.2.7 (Dex)medetomidine combined with isoflurane

Two articles investigated the temporal evolution of fc under a CRI of medetomidine (0.3 mg/kg/h, route and initial bolus not reported, on top of 1.3% isoflurane, (Nasrallah et al., 2014b)), or dexmedetomidine (0.015 mg/kg/h sc on top of 0.5-

0.75% isoflurane; initial bolus of 0.015 mg/kg ip after isoflurane induction (Brynildsen et al., 2017)) in addition to isoflurane.

Brynildsen et al. (2017) assessed connectivity to a seed in orbital frontal cortex during the first 30 min, 30-90 min and 90-150 min after start of the dexmedetomidine CRI. Spatially more extended connectivity was observed over time: while connectivity was primarily found around the seed in the first phase, significantly higher correlation with voxels in prelimbic and cingulate cortex was observed in the second phase. In the third phase, correlations were additionally significantly higher in bilateral orbital cortex, retrosplenial cortex, parietal association area, primary and secondary visual cortex and auditory cortex and temporal association area. No significant difference was found between the second and the third phase. Nasrallah et al. (2014b) observed during the first 40 min after start of the CRI a gradual, significant decrease of S1FL interhemispheric connectivity relative to that observed under isoflurane, reaching its peak 30-35 min after start of the CRI, and a significant decrease in thalamic interhemispheric connectivity, that peaked at 10-15 min after start of the CRI. In both areas, correlations in the 0.01-0.04 Hz range were significantly reduced after addition of medetomidine and for the thalamus, correlations in the 0.04-0.07 Hz range were also significantly reduced.

An additional direct comparison of combined isoflurane and medetomidine (1.5% isoflurane plus 0.45 mg/kg medetomidine bolus sc, CRI 0.2 mg/kg/h iv) with isoflurane (1.0, 1.5 and 2.0%) did not address conventional fc measures, but instead calculated degrees of freedom (DOF) of the BOLD signal (Kundu et al., 2014). A high degree of freedom was interpreted as a higher amount of structured information within the signal and thus considered desirable. DOF of the isoflurane/medetomidine combination was in the same range as observed under 2% isoflurane.

Taken together, Nasrallah et al. (2014b) report lower fc relative to previous isoflurane monoanaesthesia and Brynildsen et al. (2017) report lower fc relative to later phases of the infusion during the first 30-40 min of (dex)medetomidine infusion on top of isoflurane. Due to the different time and reference frames covered, no conclusive pattern of fc evolution can be derived. Additionally, the doses of isoflurane and (dex)medetomidine were both substantially higher in the study by Nasrallah et al. (2014b) than in the study by Brynildsen et al. (2017). The third study indicates that when combining (dex)medetomidine and isoflurane, a lower concentration of isoflurane is required to achieve a similar level of anaesthesia than when only isoflurane is used (Kundu et al., 2014).

3.4.1.2.8 Isoflurane versus ketamine/xylazine

Hutchison et al. (2010) compared 1% isoflurane to ketamine/xylazine 80/10 mg/kg ip and found no significant difference in the number of animals showing bilateral components identified by ICA per anatomic region. There was interindividual variability in component-specific power spectra, but no consistent peaks within a group and accordingly no between-group difference. Seed-based analysis was performed in a subset of both groups and bilateral connectivity between seeds and the contralateral homotopic region was observed in both groups, although again interindividual variability in fc maps and thresholds at which the interhemispheric connectivity was seen, was found.

This is the only article in this section which did not find any difference between anaesthetic protocols. The reported interindividual variability may have masked eventual between-group differences, however, without further evidence this remains speculation.

3.4.1.2.9 Medetomidine and ketamine

Two articles based on the same dataset report how a single dose of ketamine (5, 10 or 25 mg/kg sc) on top of medetomidine (0.07 mg/kg sc bolus, 0.14 mg/kg/h CRI) affects fc. The original article used a seed-based approach as well as pairwise connectivity of ROIs (Gass et al., 2014). ROI-ROI connectivity of 3 prefrontal (infralimbic, prelimbic and orbitofrontal cortex), 2 cingulate, 1 retrosplenial and 4 hippocampal ROIs was assessed by ANOVA. Significant dose effects are reported for connectivity between prefrontal and cingulate ROIs as well as some pairs on the anterior-posterior axis, with hippocampal and retrosplenial ROI on the posterior side and prefrontal and cingulate ROI on the anterior side. The time post-injection did however not have a significant effect on correlation values of any ROI pair. Seed-based analysis revealed that ketamine most strongly increased correlations in prefrontal regions for the majority of seeds. For hippocampal seeds, changes were typically stronger at 30 than at 15 min post-injection. Of the prefrontal seeds, strongest changes were observed for the seed in infralimbic cortex, which displayed increased connectivity with orbital, cingulate and prelimbic cortex as well as the postsubiculum at one or both time points after injection. Overall, ketamine increased connectivity in hippocampal prefrontal circuits, and whether changes were time-dependent depended on the method of analysis. The re-analysis confirms a significant increase of connectivity between left prelimbic cortex and both hippocampi, right prelimbic cortex and left hippocampus, but not right prelimbic cortex and ipsilateral hippocampus, after ketamine administration (Grimm et al., 2015).

A third article reports the evolution of fc over time after a single bolus of ketamine/medetomidine (60/0.5 mg/kg ip) (Bettinardi et al., 2015). 15 sliding windows (10 min per window, slided in 1 min steps) starting from 60 min post induction were defined as deep anaesthesia period and the last 15 recorded windows (from 115 min post induction on) as light anaesthesia. A significant increase of mean BOLD signal variance (for each ROI between sliding windows), mean correlation values of ROI-ROI pairs, SD of the correlation distribution, mean global synchronization (assessed by Kuramoto order parameter) and functional integration was observed in the light phase compared to the deep phase. Functional segregation (modularity) significantly decreased in the light phase. In the light, but not deep phase, five groups of robust connected nodes, i.e. pairs of areas that were consistently correlated across subjects, were identified. Over time, correlation of connected areas increased. This was not the case for unconnected areas. Overall, this study indicates more synchronized BOLD signal fluctuations at lighter levels of anaesthesia, however, trends were not for all measures monotonic.

Taken together, a bolus of ketamine, either applied on top of or in combination with medetomidine, appears to affect aspects of fc in a dose- and time-dependent way, although time-dependence was inconsistently found when different methods of analysis were used.

3.4.1.2.10 Medetomidine versus α-chloralose

Medetomidine was also compared with α-chloralose as a protocol for rsfMRI. Fc was stable over time under medetomidine (0.01 mg/kg iv bolus, 0.1 mg/kg/h iv), but increased under α-chloralose (60 mg/kg ip bolus, 30 mg/kg ip top-up every 60 min) (Paasonen et al., 2016a). Correlation between baseline fc and responses to nicotine stimulation in a phMRI was “mainly poor” under α-chloralose, and “more consistent” under medetomidine in subcortical regions.

Another study modelled rs BOLD signal fluctuations as a “scale-free (fractal) distribution of amplitude power across a frequency range” (Herman et al., 2011). Scaling exponent (fractal index) β of that distribution was interpreted as “a variable responding to physiology” and in cortical, but not subcortical ROI higher under medetomidine than under α-chloralose. How this relates to other measures of fc remains elusive.

3.4.1.2.11 Urethane

Urethane is known to produce alternating fast and slow wave states that resemble REM and NREM sleep in EEG, respectively. In rsfMRI, phases of higher and lower baseline BOLD signal were observed and assumed to represent REM- and NREM-like states, respectively (dose 1.0 g/kg iv; (Zhurakovskaya et al., 2016)). In the REM-like state, higher thalamocortical fc (between thalamus and ipsilateral somatosensory and motor cortex) than during NREM-like state was observed, while relatively higher cortico-cortical fc (between somatosensory and motor cortices) was observed during NREM-like states. In contrast, connectivity between the hippocampus and other brain regions was not state-dependent. A specific investigation of the olfactory system (dose 1.5 g/kg ip; (Wilson et al., 2011)) used respiratory rate to determine slow and fast wave states. During slow wave state, correlations on fc maps were more and ROI-ROI connectivity was significantly increased for all constellations involving piriform cortex or the dorsal hippocampus, with the single exception of connectivity between piriform cortex and amygdala. As both studies indirectly determined fast and slow wave states, it is unknown whether the defined states exactly paralleled the states known from EEG measurements. However, alternating states were clearly observed in rsfMRI and affected fc across widespread regions. Application of urethane for rsfMRI without tracking the different states is therefore likely to introduce bias.

The increase of fc over an interval of 1 h reported by Paasonen et al. (2016a) may thus reflect either measurements at two different states or a trend of increasing fc in both states over time.

3.4.1.2.12 Multiple comparisons

Finally, Paasonen et al. (2016a) report a direct comparison of isoflurane, medetomidine, α-chloralose, urethane and thiobutabarbital. Results for the isoflurane versus medetomidine, medetomidine versus α-chloralose and urethane over time comparisons were also reported in previous sections. Fc was stable over time under isoflurane (1.3%), medetomidine (0.01 mg/kg iv bolus, 0.1 mg/kg/h iv CRI) and in a subgroup of animals under thiobutabarbital (140 mg/kg ip). Fc significantly increased

over time under α-chloralose (60 mg/kg iv initial dose, 30 mg/kg iv top-up every 60 min), urethane (1.25 g/kg ip) and the second thiobutabarbital subgroup. Correlation between fc and response to phMRI with nicotine, averaged over a set of cortical regions and a set of subcortical regions, was not significant in either category under urethane and α-chloralose, significant for subcortical regions only under medetomidine, and significant for both categories under isoflurane and thiobutabarbital.

3.4.1.2.13 Summary

In summary, studies consistently report reduced fc in the anaesthetised compared to the awake state, with the limitation that isoflurane was the only anaesthetic investigated. There is a trend across anaesthetics that lower doses of isoflurane, medetomidine and propofol are associated with higher fc strength, spatially more extended connections, larger networks and/or a greater repertoire of dynamic brain states (Liu et al., 2011; Tu et al., 2011; Wang et al., 2011; Nasrallah et al., 2012; Liu et al., 2013a; Nasrallah et al., 2014a; Gill et al., 2017; Ma et al., 2017). However, this trend is neither consistently found (isoflurane: (Pan et al., 2011; Liu et al., 2013b), medetomidine: (Pawela et al., 2009)) nor monotonic (propofol: (Liu et al., 2013a)).

Some articles analysed BOLD signal amplitudes or power spectra. They consistently reported reduced amplitudes and/or power under higher concentrations of isoflurane (Wang et al., 2011; Liu et al., 2011, 2013b; Nasrallah et al., 2014a), but not medetomidine (Nasrallah et al., 2014a). Regarding power spectra, Williams et al. (2010) did not report significant differences between isoflurane and dexmedetomidine. However, longer exposure to isoflurane before switching to dexmedetomidine significantly suppressed total power in different frequency bands as well as maximum power for up to 3 hours after the switch.

A subset of articles used specific measures which, generally spoken, asses information content of the resting state BOLD signal: entropy and mutual information under different levels of isoflurane and awake (Hamilton et al., 2017); Hurst exponent at different levels of isoflurane (Wang et al., 2011); degrees of freedom at different isoflurane levels and a medetomidine/isoflurane combination (Kundu et al., 2014); Lempel Ziv complexity under different levels of propofol (Hudetz et al., 2016). Across anaesthetics they consistently report reduced information content under deeper levels of anaesthesia as well as in comparison to the awake state.

While dose matters, time also matters. Shortly after a bolus of anaesthetics, anaesthesia or sedation levels are deepest, decreasing then over time, which may be mirrored in increasing fc strength or spatially more extended connectivities over time (e.g. after a single bolus of medetomidine and ketamine (Bettinardi et al., 2015)). This effect may also explain why fc was lowest after start of a dexmedetomidine-CRI on top of isoflurane and then increased over time (Brynildsen et al., 2017). Especially for (dex)medetomidine-CRIs, however, evolution of fc over time demonstrated complex patterns. Although Paasonen et al. (2016a) observed stable fc between two resting state scans separated by 1 h, the study by Pawela et al. (2009) suggests that fc under a constant rate of medetomidine decreases when experimental times exceed 90 to 120 min, but – counterintuitively – increases when infusion rates are increased after that critical period. Considering that not only pharmacokinetics, but also the brain’s properties of a dynamic system, i.e. the potential to adapt to constant

conditions – as illustrated by Magnuson et al. (2014) – can influence resting state measurements over time, the timing of resting state measurements under a specific anaesthesia protocol is crucial.

3.4.1.3 Effects of different states of anaesthesia on BOLD responses to peripheral stimulation

Twenty references investigated the effect of states of anaesthesia on peripheral stimulation paradigms. The majority of references used an electrical paw stimulation protocol. Three references used mechanical stimulation (air puffs, whisker deflection), two references injected irritating substances into the paw and one reference investigated a visceral stimulus. Those references are discussed separately at the end of the chapter.

Unless stated otherwise, robust activation was observed in S1 contralateral to the stimulated paw in all electrical paw stimulation studies.

3.4.1.3.1 Electrical stimulation – (dex)medetomidine, isoflurane and combined (dex)medetomidine/isoflurane

Three references investigated the effect of medetomidine doses on responses to electrical paw stimulation. Nasrallah et al. (2014a) did not find a significant difference in BOLD signal increases across doses and thereby confirmed findings from a previous study of that group (Nasrallah et al., 2012), which observed no significant difference in BOLD signal increase and number of activated voxels within S1 between the same three rates of medetomidine infusion (0.1,0.2 and 0.3 mg/kg/h ip starting 40 min after a bolus of 0.05 mg/kg ip in both studies).

Pawela et al. (2009) investigated the evolution of responses to different stimulation frequencies over time under constant or increased secondary medetomidine infusion rate (0.1 mg/kg/h iv for 120 min, 0.1, 0.15, 0.2 or 0.3 mg/kg/h iv secondary infusion rate, 30 min equilibration prior to 90 min of image acquisition in both parts of the experiment). Under the initial 0.1 mg/kg/h, both the number of activated voxels and the AUC of the BOLD signal change significantly increased with higher stimulus frequencies. The frequency dependence of the number of activated voxels was significantly lower (lower slope) in the second half of the experiment than in the first when the infusion rate was left constant or increased to 0.15 mg/kg/h, whereas higher infusion rates conserved it (slope not significantly different). However, secondary infusion rates did not affect the frequency dependence of the BOLD signal intensity. That means that in prolonged experiments area of activation but not signal intensity may change over time in a stimulation frequency-dependent way when infusion rates are left constant.

Taken together, these studies present complementary evidence of no significant differences between medetomidine infusion rates at short term (40 to approximately 100 min after start of the constant rate infusion (CRI) (Nasrallah et al., 2012, 2014a)) but complex patterns after around 2 h of CRI.

In addition to medetomidine, Nasrallah et al. (2014a) also compared three concentrations of isoflurane. In contrast to medetomidine, responses to stimulation decreased under higher isoflurane concentrations. Graphs suggest that BOLD

responses to 3 mA, 9 Hz stimulation were of similar amplitude under medetomidine and 2% isoflurane, however, this finding should not be generalized to other frequencies, as frequency-dependence of the BOLD response is observed under most anaesthetics and tends to be agent-specific.

Finally, two studies added a (dex)medetomidine-CRI to constant isoflurane levels. Brynildsen et al. (2017) acquired stimulation scans every 15 min over 2.5 h after start of the dexmedetomidine-CRI. BOLD response in S1FL significantly increased after the first 30 min (phase 1) but was not significantly different between phase 2 and 3 (60 min each). No differences over time are reported for the activated areas (S1FL, S2, ventral posteromedial (VPM) and VPL nuclei of thalamus, plus cingulate and retrosplenial cortex).

Nasrallah et al. (2014b) acquired stimulation scans from 40 min after start of the CRI on and observed an increase in the area of activation under isoflurane plus medetomidine compared to the baseline under isoflurane alone, which was not observed when only the vehicle was infused. BOLD signal change under isoflurane plus medetomidine was also significantly higher than under isoflurane alone.

Regarding the time frames after initiation of the CRI, data acquired in the latter study (Nasrallah et al., 2014b) would correspond to data acquired in phase 2 of the first study (Brynildsen et al., 2017). However, as one article only reports signal intensities and localization of the activations relative to isoflurane and the other only relative to other timepoints of the dexmedetomidine “on top” infusion, results cannot be directly summarized, i.e. we do not know whether the “lower than later” response early after initiation of the dexmedetomidine-CRI would be of comparable size as the “lower than under medetomidine” response under isoflurane.

Both medetomidine and isoflurane were also compared to α-chloralose, around which a second set of references clusters. Two references compared α-chloralose with medetomidine. Sanganahalli et al. (2009) reports that while under both anaesthetics BOLD signal in S1FL strongly increased in response to stimulation, the optimal stimulation frequency (at 2 mA) differed between anaesthetics: under α-chloralose (46 +/- 4 mg/kg/h ip) it was 3 Hz and under medetomidine (0.1 mg/kg/h ip) 9 Hz. Accordingly, amplitudes and shape of the BOLD signal significantly differed between anaesthetics at 3 and 9 Hz stimulation frequency. For the other frequencies between 1 and 24 Hz which were also investigated no results are explicitly reported (limited detail of results because reference is a conference abstract). Inter- and intra-subject reproducibility of BOLD responses was reportedly higher under α-chloralose.

In contrast, Weber et al. (2006) stimulated animals with 3 Hz at the same intensity as Sanganahalli et al. (2009) and did not find a significant difference in BOLD signal increase or the area of activation in S1 between sessions under medetomidine (0.05 mg/kg sc bolus followed by 0.1 mg/kg/h sc) or α-chloralose (50 mg/kg iv initial dose, 36 mg/kg iv top-up every 60 min). One out of four animals showed additional activation in S2 under medetomidine and two out of three under α-chloralose. Time from bolus administration under halothane until a response to stimulation could be measured was longer under medetomidine (81 +/- 10 min) than under α-chloralose (61 +/- 3) with the doses used in that study.

Taken together, conflicting results were found as to whether signal increase in S1 was different under medetomidine and α-chloralose. Other findings presented in the two studies available were complementary.

3.4.1.3.2 Electrical stimulation – α-chloralose versus various

One study found higher signal intensity (significantly larger area under the curve) during the first, but not second of two stimulation periods under α-chloralose (80 mg/kg iv bolus, 40 mg/kg/h iv CRI) than under isoflurane (1.2%) (Sommers et al., 2009). Localization of the activation did not differ between anaesthetics; in both conditions it was centred in S1. A conference poster reports for two animals a 10% lower baseline BOLD signal, but higher responses to forepaw stimulation from 80 to 90 min on after anaesthesia was changed from isoflurane (2%) to α-chloralose (50 mg/kg iv bolus, 40 mg/kg/h iv CRI) (Gsell et al., n.d.). Higher BOLD signal response was thus consistently reported under α-chloralose at different doses of both isoflurane and α-chloralose.

Furthermore, some studies compared α-chloralose to currently less commonly used anaesthetics.
Compared to halothane (1%), responses to forepaw stimulation were more localized and tended to be stronger (no information on significance provided) under α-chloralose (45 +/-9 mg/kg/h ip) (Maandag et al., 2007). While activations in regions other than S1 spread to ipsilateral and posterior regions under halothane (including lateral areas of the hippocampus (H) as well as some secondary areas of the visual (V) and auditory (A) cortices), they were limited to contralateral S2 and M1/M2 under α-chloralose. Activation patterns outside S1 were more reproducible under α-chloralose and activation in S1 detected more consistently (“nearly all” versus “majority of the experiments”).

In comparison to pentobarbital (143-173 mg/kg ip), α-chloralose (50 mg/kg ip) was reported to produce a “tighter” cluster of activated voxels in S1 (visually only small cluster under pentobarbital) and upon visual inspection signal fluctuations corresponded more clearly to the stimulus time course under α-chloralose (Kuo et al., 2005). Frequency-dependence of the BOLD signal was observed under both anaesthetics and under both, highest signal intensities were obtained with 3 Hz stimulation frequency. Except at 3 Hz, where responses were significantly higher under α-chloralose, no significant differences in signal intensities were found.

Finally, comparison with urethane (1.25 g/kg ip) revealed anaesthetic-specific frequency-dependencies of the BOLD responses to paw stimulation (Huttunen et al., 2008). BOLD signal intensities were significantly different at all stimulation frequencies; while under α-chloralose (60 mg/kg iv, 30 mg/kg iv top-up after 60 min) responses were only observed at 1 and 3 Hz, they were present only at 3 Hz and higher under urethane. Localization of the responses exclusively in S1 and centre of the activation did not differ between conditions.

In summary, single reports suggest an overall more favourable profile of BOLD responses to paw stimulation under α-chloralose than under halothane and pentobarbital and a range complementary to urethane of stimulation frequencies for which a response can be observed.

3.4.1.3.3 Electrical stimulation – awake versus anaesthetised

Peeters et al. (2001) first imaged paralysed awake animals, then the same animals under α-chloralose anaesthesia. Paralysed animals showed higher volumes of

activation both in contralateral S1 and the less specific ROIs of contra- and ipsilateral somatosensory cortex, as well as a trend for higher signal intensities, however, localization of the activations was more variable.

In a cross-over of propofol and the awake state, Lahti et al. (1999) found significantly higher signal increase in cortical areas and confirmed the regular occurrence of activity in the ipsilateral somatosensory cortex in the awake state.

Stronger signal increase or larger areas of activation, but less specific localization than under anaesthesia were thus consistently reported for awake imaging.

3.4.1.3.4 Mechanical stimulation

Instead of electrical stimulation, Chang et al. (2016) used innocuous air puffs to the side of the hindpaw when comparing responses to stimulation in trained awake and isoflurane-anaesthetised animals. While significant positive signal changes were observed in bilateral S1HL, thalamus and cingulate cortex, and negative signal changes in bilateral S2, hypothalamus and ipsilateral insular cortex upon stimulation in awake animals, only when significance thresholds were lowered, activation in S1HL could be detected under 3% isoflurane. Correspondence of the signal time course with the stimulus time-course was visually more obvious in awake animals.

Dashti et al. (2005) also used air puffs, but over the eye, to characterize BOLD responses under isoflurane (1.6%) and equithesin (6.5g chloral hydrate, 1.44g pentobarbital, 2.7g magnesium sulphate, 15 ml alcohol, 52.5 ml propylene glycol). Under equithesin, only 1 out of 16 stimulation scans showed activation in somatosensory cortex, whereas activation was consistently detected under isoflurane.

Additionally, de Celis Alonso et al. (2011) compared responses to whisker deflection in a longitudinal study of two sessions each of isoflurane and α-chloralose. Numbers of activated voxels (in S1, S2 and VPM nucleus of the thalamus) were significantly lower in both isoflurane scans than in both α-chloralose scans. Signal intensity in all three regions was significantly lower in the first, but not in the second isoflurane scan compared to both α-chloralose scans. Generally, activations were more widespread under α-chloralose, with activations also detected in CPu, retrosplenial cortex and ipsilateral S1.

Taken together, two studies inconsistently report the presence of BOLD signal responses to air puff stimulation under isoflurane, while responses to whisker deflection were observed under isoflurane, but weaker and spatially more localized than under α-chloralose.

3.4.1.3.5 Chemical somatosensory stimulation

Another two studies injected irritating substances into the paw under different doses of isoflurane or comparing isoflurane with α-chloralose.

Setting observation of both an early and a late response to formalin injection as the criterion, Asanuma et al. (2008) found that both 1.0 and 1.2% isoflurane fulfilled it, but not 0.6, 0.8 and 2.0% (animals were ventilated and paralysed at all concentrations). Chen et al. (2008) briefly report weaker BOLD responses to formalin

injection under a not specified concentration of isoflurane than under 70 mg/kg ip α-chloralose. This relation was conserved when animals were pre-treated with morphine. As responses under isoflurane appear to be dose-dependent, but the isoflurane concentration is not reported in the latter study, and different outcome measures were used, it is unclear whether results of the two studies were consistent.

3.4.1.3.6 Visceral stimulation

Finally, one reference analysed responses to visceral stimulation. This study compared α-chloralose, isoflurane and awake imaging regarding effects on BOLD signal responses to intragastric L-Glutamate administration (Tsurugizawa et al., 2010). Positive BOLD signal change was observed in a variety of – mainly shared – regions under the awake and α-chloralose-anaesthetised condition, but only in a few regions under isoflurane. In contrast, negative BOLD signal changes were commonly observed under isoflurane, but only sporadically under α-chloralose and not in awake animals.

3.4.1.3.7 Summary

References investigating responses to peripheral stimulation used a variety of stimulation modalities and anaesthetic protocols, resulting in maximally 3 references per comparison of interest and modality. If findings are summarized across stimulation modalities, α-chloralose appears to produce an overall more desirable profile of responses than isoflurane, although in which parameters exactly responses are superior to those acquired under isoflurane varies. The most commonly mentioned parameter is signal intensity, which was typically higher if α-chloralose was used (Chen et al., 2008; Gsell et al., n.d.), although two studies also found this difference only in some runs (Sommers et al., 2009; de Celis Alonso et al., 2011). Two studies reported activation in more extended areas under α-chloralose than isoflurane (Tsurugizawa et al., 2010; de Celis Alonso et al., 2011), while another reported that the centre of activation was not different (Sommers et al., 2009). Across stimulation modalities, activations were observed in more extended regions in awake animals than in isoflurane- or propofol-anaesthetised animals (Lahti et al., 1999; Tsurugizawa et al., 2010; Chang et al., 2016), while findings in comparison to α-chloralose were inconsistent (Peeters et al., 2001; Tsurugizawa et al., 2010), which may or may not be related to the different stimulation modalities used in those studies.

Overall, the heterogeneity of the data prevents strong conclusions.

3.4.1.4 Effects of different states of anaesthesia on BOLD responses to central stimulation

Eleven references compared BOLD responses to central stimuli between different states of anaesthesia. Seven applied pharmacological or chemical stimuli, three used direct electrical stimulation of brain regions and one reference used optogenetic stimulation. Results are summarized for each stimulation modality separately.

3.4.1.4.1 Pharmacological/chemical stimulation

Two references report comparisons of multiple anaesthetics for pharmacologic MRI (phMRI). One study compared medetomidine (0.1 mg/kg iv bolus, 0.1 mg/kg/h iv CRI), isoflurane (1.3%), α-chloralose (60 mg/kg iv, top-up 30 mg/kg iv after 60 min), urethane (1.25 g/kg ip) and thiobutabarbital (TBB, 140 mg/kg ip) for nicotine phMRI (Paasonen et al., 2016b). The urethane and thiobutabarbital groups were each divided into a mechanically ventilated and spontaneously breathing group. In all other groups, animals were ventilated. ROIs were defined both by summarising ICA components with high signal time course correlations and in reference to an anatomic atlas. With the ICA approach, activation was detected in similar regions across groups. As in a subset of ventilated TBB animals mainly negative signal changes were observed, the ventilated TBB group was further divided in two subgroups (TBB1 positive, TBB2 negative signal changes). Considerable variability was observed in anatomically defined ROI both between regions and anaesthetics. In subcortical regions, no significant difference was found between anaesthetics. In cortical regions, area under the curve of the signal time course was significantly higher in both urethane groups than under all other anaesthetics, under TBB1 than under α-chloralose and isoflurane, and under medetomidine than under α-chloralose.

The other study investigated activations after administration of two different doses of levo-tetrahydropalmatine (l-THP) under medetomidine (0.1 mg/kg sc bolus, 0.1 mg/kg/h iv CRI), isoflurane (1.4-1.6%) and urethane (1.2 g/kg ip) (Liu et al., 2012). Activation was defined as AUC of percent signal change of individual voxels compared to injection of saline. ANOVA revealed significant effects of l-THP dose and anaesthetic agent in specific, partially overlapping regions (anaesthetic alone: cingulate cortex, insula, hypothalamus, CPu, globus pallidus, amygdala, retrosplenium, parietal cortex, ventral tegmental area, and raphe nucleus) and widespread l-THP dose-anaesthetic interaction effect in 15 regions (largest clusters in hippocampus and somatosensory cortex). Under the lower dose of 5 mg/kg l-THP, least extended, consistently positive activations were observed under isoflurane, more extended, mostly positive activations under medetomidine, and mostly negative activations under urethane. The higher dose of 20 mg/kg l-THP elicited more extended activations under all anaesthetics and added some negative responses under isoflurane. The highest number of areas was activated under urethane, but those were exclusively negative activations.

Taken together, while Paasonen et al. (2016b) found strongest positive BOLD responses under urethane, Liu et al. (2012) observed only negative responses under urethane. However, due to the different stimulation drugs for phMRI, findings are complementary rather than contradictory.

Awake imaging was compared to medetomidine sedation (Airaksinen et al., 2012) and isoflurane anaesthesia (Tenney et al., 2003) in two different epilepsy models. Airaksinen et al. (2012) observed in a kainate model that not all electrophysiologically detected seizure activity was accompanied by BOLD signal changes. In the awake group, seizures which were accompanied by a BOLD response were significantly longer than those which were not accompanied by a response, whereas such a relation was not evident under medetomidine (0.05 mg/kg sc bolus, 0.1 mg/kg/h sc CRI). The occurrence of seizures as well as BOLD responses, was however not significantly different between groups, and localization of the activations was also similar, leading to the authors' conclusion that medetomidine was a suitable sedative

to study seizure activity in the kainate model. Tenney et al. (2003) briefly report that BOLD responses were “dramatically reduced” under 2% isoflurane in comparison to awake animals in the γ-butyrolactone model of absence seizures. Activation maps show a difference in spatial extent of BOLD responses, but the text does not specify which aspects of the BOLD response were reduced.

Taken together, BOLD responses during seizures were higher than or similar to those observed under anaesthesia in awake animals in two different models of epilepsy.

Three references studied activations after a single bolus of ketamine in isoflurane anaesthetised animals. Littlewood et al. (2006b) compared the responses to two subanaesthetic doses of ketamine (10 or 25 mg/kg sc) with responses to saline under 1.5% isoflurane. BOLD signal time courses in specific regions were correlated with locomotor activity observed in awake animals over time at the corresponding doses and significant activations were observed in “frontal, hippocampal, cortical and limbic areas”. At the higher dose of ketamine, some negative activation (in the inferior colliculus) was also found. Input functions of the high and low doses were exchanged to demonstrate dose-dependent effects. When the locomotor input function of 10 mg/kg ketamine was used for phMRI of both doses, activations in limbic and cortical areas appeared significantly stronger under 25 mg/kg and 10 mg/kg, respectively. Dose-dependencies were stronger when results of microdialysis (from ventral pallidum and nucleus accumbens) were used as the input function instead of the locomotor input function. Signal time courses after ketamine and vehicle injection differed only in selected ROIs. No significant difference was found in whole brain signal intensity.

Another study from the same group administered racemic ketamine, the S-enantiomer or the R-enantiomer (25 mg/kg each), or only the vehicle on top of 1.6% isoflurane and observed for racemic and S-ketamine overall similar activations, especially in limbic regions, although cortical and hindbrain activations were less pronounced after S-ketamine (Littlewood et al., 2006a). Again, differences in signal time courses between racemic or S-ketamine and vehicle were observed only in ROI which were activated in statistical parametric mapping (SPM) maps.

A third study investigated the interactions of TAK-063, a potential anti-psychotic, and ketamine, which is commonly used as a model for schizophrenia (Tomimatsu et al., 2016). Ketamine (10 mg/kg sc) alone on top of 1.5-1.8% isoflurane induced positive BOLD signal changes in cortex, hippocampus, CPu and amygdala and negative signal changes in brainstem, cerebellum and hypothalamus. Previous administration of TAK-063 prevented negative signal changes, decreased the positive signal change in cortical and hippocampal areas and led to stronger positive activation in the CPu. At the lower of two doses, TAK-063 additionally increased signal in thalamus and hypothalamus.

Taken together, all three studies consistently report activation in cortex, hippocampus and variable other regions after administration of a subanaesthetic dose of ketamine or S-ketamine.

3.4.1.4.2 Electrical stimulation

Three references compared anaesthetic protocols for direct electrical brain stimulation.

Austin et al. (2005) implanted two electrodes subdurally over left hindpaw motor cortex and compared BOLD responses to stimulation under different concentrations of halothane (0.7, 0.8, 0.9, 1.0, 1.2, 1.5%) plus N_2O and at different timepoints after switch to α-chloralose. Under all halothane concentrations BOLD responses in the ipsi- and contralateral motor cortex were observed without significant differences in area of activation between concentrations. Independent of concentrations, activations in additional regions, for example S2, were observed in some animals. Amplitude of the BOLD response did not significantly differ across concentrations and the duration of the response was only at 1.5% halothane and only in the stimulated cortex significantly longer than under other concentrations. Under α-chloralose, activations were for the first 2 h also limited to bilateral motor cortices, later, however, significant activations in other cortical and subcortical areas were observed. The activated area within the motor cortices was 1 h after the initial bolus lower than under halothane but grew larger over time 2-6 h after the initial bolus (significant relative to 1 h from 3 h on in both cortices). Throughout the experiment, responses were slower under α-chloralose than under halothane (significant difference to halothane at all timepoints), although the latency decreased over time. Similar trends for increased latencies after change to α-chloralose were observed in secondary activated regions.

In contrast, Lai et al. (2015) and Chao et al. (2014) both studied deep brain stimulation. Lai et al. (2015) stimulated VPM at frequencies of 1, 2, 5, 10, 15, 20, 25 and 30 Hz. At 10, 15 and 20 Hz, signal amplitudes in somatosensory cortex were significantly higher under α-chloralose (60 mg/kg iv bolus, 30 mg/kg/h iv CRI) than under isoflurane (1.0-1.25%). Under both anaesthetics, however, robust responses were observed, which were highest at 20 Hz and higher at 10-20 Hz than at 1Hz.

Chao et al. (2014) found frequency and intensity dependent BOLD responses in S1 upon stimulation of ventroposterior thalamus (VP) under dexmedetomidine (0.025 mg/kg sc bolus, 0.05 mg/kg/h sc CRI), but stable signal time courses and localization of the activation across sessions under the same frequency/intensity combination. In contrast, signal amplitudes and number of activated voxels in S1 varied considerably between sessions with identical stimulation parameters in isoflurane-anaesthetised (1.0-1.3%, adjusted according to depth of anaesthesia as assessed by respiratory rate) animals.

Taken together, while one reference found robust activation in S1 under isoflurane (Lai et al., 2015) the other observed highly variable activations at the same concentration (Chao et al., 2014). However, electrode location, stimulus waveforms (bi- versus monophasic square waves), current intensities (2 mA versus 0.1-0.3 mA) and impulse durations (1/f ms, i.e. 0.1 ms at 10 Hz, versus 0.4 ms) differed between studies, indicating that responses under isoflurane depend on stimulation parameters. On the other hand, both a deep brain stimulation (Lai et al., 2015) and a subdural stimulation study (Austin et al., 2005) found robust activations in the expected regions under α-chloralose within the first 2 h after the initial bolus.

3.4.1.4.3 Optogenetic stimulation

Finally, one study used an optogenetic approach to stimulate the infralimbic part of medial prefrontal cortex in awake animals and under isoflurane (1.0-1.15%) (Liang et al., 2015b). Activations were spatially more extended and had a higher BOLD signal amplitude in awake animals. Awake animals showed activations in several cortical and subcortical areas. This pattern was reproducible within and consistent across animals. In contrast, under isoflurane activations were observed only around the fibre tip, in insula and orbital cortex. Signal intensities in activated regions ranged from 2% to 3-4% during stimulation in awake animals and never exceeded 1% in anaesthetised animals (standard errors of the means not overlapping in graphic).

3.4.1.4.4 Summary

Taken together, electrical brain stimulation yielded robust responses under α-chloralose whereas robust responses were inconsistently found under isoflurane. Responses to subanaesthetic doses of ketamine were overall similar between studies, seizure activity was detected with variable success under different anaesthetics/sedatives in different epilepsy models, and differences specific for the tested substance were observed in phMRI when comparing multiple anaesthetic protocols.

3.4.1.5 Effects of different states of anaesthesia on baseline BOLD signal

Studies which could not clearly be allocated to one of the previous experimental paradigms are described in this section containing three references which all investigated some version of baseline BOLD signal under different states of anaesthesia, typically with the aim to understand how the BOLD signal relates to other measures.

Gong et al. (2014) observed BOLD signal changes in three layers of olfactory bulb under 1.8 and 3.5% isoflurane. In all layers (glomerular, mitral and granulous cell layer), BOLD signal increased when the isoflurane concentrations increased and decreased when the isoflurane concentration was decreased – the contrary of what was observed for local field potentials (LFP). Additionally, significant differences in the magnitude of signal changes were found between layers (which was also not the case for LFP).

Tsurugizawa et al. (2016) used high resolution imaging to investigate the effect of different isoflurane concentrations (1.5, 2.0, 2.5, 3.0%) on BOLD signal originating from capillaries (called “tissue” BOLD signal) and large arteries and veins (called “vessel” BOLD signal) in somatosensory cortex. In somatosensory cortex as a whole, BOLD signal was significantly higher at 2.0 and 2.5% isoflurane than at 1.5% and decreasing again at 3.0%, resulting in an inversed U-shape. The same pattern was observed when only tissue or vessel areas of the ROI were analysed, however, vessel BOLD signal at 2.5% isoflurane was significantly higher than tissue BOLD signal.

Abe et al. (2017) investigated relations between the BOLD signal and LFP as opposed to apparent water diffusion coefficient (ADC) and LFP under two doses each of isoflurane (1.5 and 2.5%) and medetomidine (0.05 mg/kg sc bolus, CRI 0.1

mg/kg/h for 40 min, then 0.3 mg/kg/h iv). While baseline BOLD signal increased with higher isoflurane dose (significantly higher at 2.5 than 1.5% in most areas), it decreased with the higher medetomidine dose (significantly lower at 0.3 mg/kg/h than at 0.1 in most areas).

Studies thus consistently report an increase in baseline BOLD signal when isoflurane concentration is increased from 1.5 to 2.5% (Tsurugizawa et al., 2016; Abe et al., 2017), but disagree whether the trend continues in higher ranges (1.8-3.5% (Gong et al., 2014)) or is reversed (Tsurugizawa et al., 2016). The findings of Abe et al. (2017) suggests that this increase is not limited to the regions studied by Tsurugizawa et al. (2016) and Gong et al. (2014) and that this signal increase is a specific feature of isoflurane, as it was not observed under medetomidine.

3.4.1.6 Conclusion

3.4.1.6.1 Awake versus anaesthetised

Imaging of awake animals was compared to results obtained under isoflurane, α-chloralose, propofol and medetomidine in various stimulation paradigms, and to rsfMRI results obtained under isoflurane. Stronger BOLD response to hypercapnia was observed in awake animals than under isoflurane, but responses to severe hypoxia were more pronounced under isoflurane. Signal intensity upon peripheral electrical stimulation was higher in awake animals than under propofol. Compared to α-chloralose, signal intensity was similar in awake animals, but area of activation was higher and spatial reproducibility lower in awake animals. When mechanical peripheral stimulation was applied, both the presence of activations in the respective area of S1 and the number of additionally activated regions were higher in awake animals than under isoflurane. The total number of activated regions was also higher in awake animals than under isoflurane in a visceral stimulation paradigm, but similar to under α-chloralose-anaesthesia. In the same study, negative signal changes were more common under isoflurane than under α-chloralose and under α-chloralose than in awake animals. In two different epilepsy models, awake imaging proved equivalent to medetomidine regarding occurrence and localization of activations during seizures, and superior to isoflurane in the general term of “BOLD response” during seizures (not defined which aspects of response). Finally, one optogenetic study reports a higher number of activated regions in awake animals upon light stimulation. Across stimulation modalities, stronger responses – in terms of signal intensity and/or activated area both in “expected” and additional regions – were thus commonly (Lahti et al., 1999; Peeters et al., 2001; Brevard et al., 2003; Sicard et al., 2003; Tenney et al., 2003; Liang et al., 2015b; Chang et al., 2016;), but not consistently (Tsurugizawa et al., 2010; Airaksinen et al., 2012) found in awake animals. Only one article reported a higher amplitude of signal change under isoflurane (Duong, 2007), but this was at the same time the only article in which stimulation resulted in a BOLD signal decrease.

Resting state studies consistently report reduced fc under isoflurane compared to the awake state based on three datasets (Liang et al., 2012a; Liang et al., 2013; Liang et al., 2015a; Chang et al., 2016; Hamilton et al., 2017; Ma et al., 2017; Smith et al., 2017). Generally, both responses to stimulation and fc were stronger in awake than in anaesthetised animals (nine of eleven datasets).

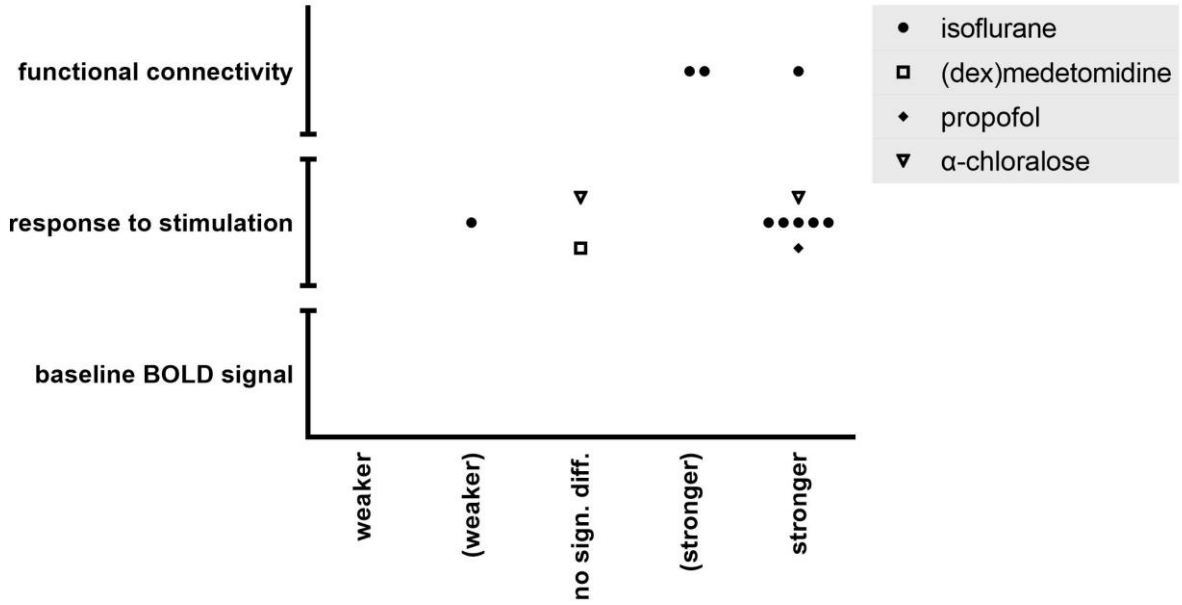

Figure 12. Comparison of response to stimulation and fc in awake versus anaesthetised animals (no data available for baseline BOLD signal). stronger = higher signal intensity upon stimulation and/or spatial extent of activated area in awake animals or higher fc strength and/or spatial extent of connectivities; (stronger) = response to stimulation or fc stronger in some, but not all aspects in awake animals; no sign. diff. = no significant difference between awake and anaesthetised. “weaker” and “(weaker)” analogous to “stronger” and “(stronger)”. One data point per dataset and anaesthetic. If re-analysis found a significant difference only in some aspects, “(stronger)” was selected.

3.4.1.6.2 Dose effects

Across anaesthetics and experimental paradigms, dose-dependence of BOLD results was a common, but not universal phenomenon. Baseline BOLD signal increased (Gong et al., 2014; Abe et al., 2017) or followed an inverse U-shape (i.e. intensity

decreasing again after a peak was reached (Tsurugizawa et al., 2016)) when isoflurane concentrations were increased, whereas higher medetomidine doses decreased baseline BOLD signal intensity (Abe et al., 2017). While medetomidine infusion rates did not affect responses to peripheral electrical stimulation within the first two hours (Nasrallah et al., 2012, 2014a), frequency-dependence of responses was partially dose-dependent thereafter (Pawela et al., 2009). Amplitude of the BOLD signal response to peripheral electrical stimulation decreased with higher isoflurane concentrations (Nasrallah et al., 2014a). In contrast, early as well as late response to sc formalin injection was only observed at intermediate (within a range of tested) isoflurane concentrations (Asanuma et al., 2008). Dose-dependence of the spatial pattern and presence of negative signal changes was also observed when ketamine or S-ketamine were administered at subanaesthetic doses on top of isoflurane (Littlewood et al., 2006b). However, neither area of activation within the motor cortex, nor the pattern of additionally activated regions, nor amplitude of the BOLD signal differed across a range of halothane concentrations combined with a constant concentration of N_2O when motor cortex was stimulated with subdural electrodes (Austin et al., 2005).

In rsfMRI there was a trend across anaesthetics that lower doses of isoflurane, medetomidine and propofol are associated with higher fc strength, spatially more extended connections, larger networks and/or a greater repertoire of dynamic brain states (Tu et al., 2011; Wang et al., 2011; Liu et al., 2011, 2013a; Nasrallah et al., 2012, 2014a; Gill et al., 2017; Ma et al., 2017;)). However, this trend was neither consistently found (isoflurane: (Liu et al., 2013b; Pan et al., 2011), medetomidine: (Pawela et al., 2009)) nor monotonic (propofol: (Liu et al., 2013a)).

Taken together, dose-dependence was a common finding, but the direction of effects was sometimes controversial.

Figure 13. Dose dependence of baseline BOLD signal, responses to stimulation, and fc in rats. No = no dose dependent effect was observed; partial = dose dependence was observed for some outcomes, analyses or animals, but not consistently; yes = a dose dependent effect was clearly observed. One point per dataset per anaesthetic. If original article and re-analysis disagreed, partial dose dependence was selected.

3.4.1.6.3 Pairwise comparison of anaesthetics across experimental paradigms

Specific comparisons of anaesthetics re-occurred across experimental paradigms and findings are summarized in the following section.

Isoflurane versus α-chloralose

Baseline signal was in a single report observed to be lower under α-chloralose than under isoflurane in two animals (Gsell et al., n.d.). The same report also found higher change under α-chloralose in signal intensity upon electrical forepaw stimulation. This finding was partially confirmed by a larger study (Sommers et al., 2009) which found in the first, but not second of two stimulation periods significantly larger AUC of the signal intensity under α-chloralose than under isoflurane, without a difference in localization of the activation. Mechanical peripheral stimulation resulted in one out of two scans in higher amplitude of the signal intensity and in two out of two scans in higher numbers of activated voxels within defined regions under α-chloralose (de Celis Alonso et al., 2011). The number of additionally activated regions was also higher under α-chloralose. Another study reported stronger BOLD response to sc formalin injection under α-chloralose than under isoflurane without further specifying the term BOLD response (Chen et al., 2008). In line with the findings reported so far, fewer regions were activated under isoflurane than under α-chloralose when a visceral stimulus was applied. However, more extended negative signal changes were observed under isoflurane than under α-chloralose (Tsurugizawa et al., 2010).

Deep brain stimulation supports the observation of tendentially stronger and/or more extended responses to stimulation under α-chloralose than isoflurane. Signal amplitudes were higher under some, but not all stimulation frequencies under α-chloralose (Lai et al., 2015). Nevertheless, under both anaesthetics, responses were robust and followed the same patterns of frequency dependence. In contrast, no significant difference between α-chloralose and isoflurane was found in AUC of the signal intensity in nicotine phMRI (Paasonen et al., 2016b). Re-analysis of parts of the same dataset regarding fc was the only information available on how α-chloralose and isoflurane compare in rsfMRI (Paasonen et al., 2016a). Correlation of region-specific baseline fc with phMRI response was assessed by the authors as high under isoflurane and “mainly poor” under α-chloralose.

In summary, there is a trend across peripheral and central stimulation modalities that fMRI under α-chloralose yields higher amplitudes or AUC of BOLD signal intensities and/or spatially more extended activations, both in expected and additional regions. For baseline and rsfMRI, the available information was insufficient to detect any trends (one reference each).

Medetomidine versus α-chloralose

Inconsistent results are reported for responses to electrical paw stimulation under medetomidine compared to α-chloralose. While one reference (Weber et al., 2006) found no difference in the change of signal intensity or the activated area at stimulation with 3 Hz (the only difference was the time between bolus administration and the first successful measurement of a response to stimulation, which was longer under medetomidine), another (Sanganahalli et al., 2009) observed peak responses at different stimulation frequencies for medetomidine and α-chloralose (9 and 3 Hz, respectively) and accordingly significantly different signal amplitudes at those (higher under medetomidine at 9 Hz, higher under α-chloralose at 3 Hz).

In a nicotine phMRI study, AUC of the signal intensity was in cortical regions significantly higher under medetomidine than α-chloralose, but not significantly different between anaesthetics in subcortical regions (Paasonen et al., 2016b). Re-analysis of parts of the same dataset regarding fc retrieved “more consistent” correlation of baseline fc with responses to nicotine, primarily in subcortical regions, under medetomidine than under α-chloralose. Finally, one reference assessed scaling exponent β and found that values were in cortical, but not subcortical regions higher under medetomidine than under α-chloralose (Herman et al., 2011).

In summary, there was no consistent trend in responses to stimulation and not enough data to detect a trend for rsfMRI.

Urethane versus α-chloralose

Responses to electrical paw stimulation were observed at different, partially overlapping frequency ranges for urethane (3-15 Hz) and α-chloralose (1-3 Hz), but not quantitatively compared where they overlapped (Huttunen et al., 2008). In phMRI, AUC of the signal intensity after nicotine administration was significantly higher under urethane than under α-chloralose in cortical regions, but no significant difference was found in subcortical regions (Paasonen et al., 2016b). When parts of the previous dataset were re-analysed, no correlation was observed under urethane between the response to nicotine and baseline fc, and “mainly poor” correlations under α-chloralose (Paasonen et al., 2016a).

Taken together, the information available is too sparse to identify a general pattern.

Halothane versus α-chloralose

Responses to electrical paw stimulation were more localized and reproducible under α-chloralose than under halothane when measured 2 h after switching from halothane to α-chloralose (Maandag et al., 2007). For superficial direct electrical stimulation of the brain, it depended on the time elapsed since the initial α-chloralose bolus how responses compared to those observed under halothane (Austin et al., 2005). One hour after the bolus, area of activation in the stimulated region was significantly smaller under α-chloralose than under halothane, however, the area of activation increased significantly over time. As for activation in additional areas, significantly more additional regions were activated under α-chloralose than under

halothane from 2 h post bolus on. Only latency of the BOLD response was significantly higher under α-chloralose than halothane throughout the experiment.

Taken together, response to stimulation may be more localized under α-chloralose under certain circumstances (e.g. dose, timing). No information was found for rsfMRI for the comparison in question.

Barbiturates versus α-chloralose

Response to electrical paw stimulation was significantly higher under α-chloralose than under pentobarbital only at one of four applied stimulation frequencies (Kuo et al., 2005). In nicotine phMRI, AUC of signal intensities was significantly higher in a subgroup of thiobutabarbital-anaesthetised animals than in α-chloralose-anaesthetised animals, but only in cortical, not in subcortical regions (Paasonen et al., 2016b). Re-analysis of the data with a focus on fc found “high and consistent” correlations under thiobutabarbital as opposed to “mainly poor” correlations of region-specific baseline fc with response to nicotine under α-chloralose (Paasonen et al., 2016a).

Taken together, although in both data sets differences in opposite directions (once stronger response under α-chloralose, then under barbiturates) were observed in subgroups, responses to stimulation appeared to be in an overall comparable range. Again, only information from single reports was available on rsfMRI.

Isoflurane versus (dex)medetomidine

In deep brain stimulation, reproducibility of signal amplitude, area of activation and localization of activations were higher under dexmedetomidine than under isoflurane (Chao et al., 2014). One phMRI study found more extended activations and mixed positive and negative signal changes under medetomidine, while under isoflurane more localized, primarily positive signal changes were observed (Liu et al., 2012). Another phMRI study however did not find a significant difference in AUC of signal intensity between medetomidine and isoflurane (Paasonen et al., 2016b).

In re-analysis of parts of the same data set, region-specific correlation values of baseline connectivity with response to nicotine were high in both groups, especially in cortical regions under isoflurane and subcortical regions under medetomidine (Paasonen et al., 2016a). Two more studies found lower and more localized correlations in seed maps under medetomidine and higher correlations in pairwise ROI analysis, which were not limited to homotopic bilateral ROI, under isoflurane (Williams et al., 2010; Kalthoff et al., 2013). In ICA incidence of cortical and striatal networks was higher and networks showed more consistent segregation patterns under medetomidine (Kalthoff et al., 2013), but no difference was found in power spectra of the BOLD signal (Williams et al., 2010). An abstract also noted “generally higher” inter-and intrahemispheric connectivity under isoflurane (Boonzaier et al., 2017).

Taken together, there was no common pattern of differences between medetomidine and isoflurane in responses to stimulation across and even within stimulation modalities. In contrast, lower and more localized correlations both between a seed and other voxels and between ROI pairs were consistently observed under medetomidine and interpreted as more specific fc.

Isoflurane versus isoflurane/(dex)medetomidine

While the activated area became more extended and the amplitude of signal significantly increased after addition of a dexmedetomidine-CRI to isoflurane, in the same study interhemispheric S1FL and thalamic connectivity significantly decreased and correlations in the 0.01-0.04 Hz range were significantly reduced in both areas (Nasrallah et al., 2014b). A second study found that the degrees of freedom of the BOLD signal under combined isoflurane/medetomidine (1.5% isoflurane plus 0.45 mg/kg sc bolus, 0.2 mg/kg/h iv CRI medetomidine) equalled those observed under 2% isoflurane (Kundu et al., 2014).

As limited data is available per experimental paradigm, general trends or ”signature patterns” cannot be identified.

Isoflurane versus urethane

In phMRI, urethane provided significantly higher AUC of signal intensity than isoflurane in cortical regions (Paasonen et al., 2016b) and higher spatial extent of responses (Liu et al., 2012). It appears thus that the magnitude of responses tends to be higher under urethane, however, the direction of signal change was positive in one study (Paasonen et al., 2016b) but negative in the other (Liu et al., 2012), whereas those observed under isoflurane were mainly positive in both studies.

When parts of the first dataset were re-analysed with a focus on fc, no correlation between baseline fc and response to phMRI was observed under urethane in contrast to isoflurane (Paasonen et al., 2016a).

(Dex)Medetomidine versus urethane

Responses to pharmacological stimulation measured as area under the curve of signal intensity changes were stronger under urethane than under medetomidine in two studies. However, while one study found primarily positive signal changes under urethane (Paasonen et al., 2016b), negative signal changes dominated in the other (Liu et al., 2012). For medetomidine, both studies report mostly positive signal changes. The areas in which activations were seen were at least in one study similar between urethane and medetomidine (Paasonen et al., 2016b).

Fc was stable over time under medetomidine and a correlation found in subcortical regions between fc and response to nicotine (Paasonen et al., 2016a). In contrast, neither temporally stable fc nor a correlation with responses to pharmacological stimulation was found under urethane.

However, as limited data is available and interactions between anaesthetic and test substance are to be expected in phMRI, generalization of these observations is not warranted.

3.4.1.6.4 Time effects

Responses to stimulation were in the first phase after administration of a bolus weaker than in later phases – despite maintenance of anaesthesia with a CRI or top-up boli at a lower dose – in terms of signal intensity (Brynildsen et al., 2017) or area

of activation (Austin et al., 2005). Sometimes responses to stimulation were completely suppressed for 60 to 80 minutes after the initial bolus of medetomidine or α-chloralose (Weber et al., 2006; Gsell et al., n.d.).

Likewise, fc strength increased or connectivities became spatially more extended after administration of a bolus of dexmedetomidine or α-chloralose (Bettinardi et al., 2015; Paasonen et al., 2016a; Brynildsen et al., 2017). However, the same was not true after administration of a subanaesthetic ketamine bolus, where differences in both directions were observed between two timepoints of measurement, depending on the region and the method of analysis.

When anaesthesia was maintained over longer periods, for example with a medetomidine-CRI, patterns became more complex (Pawela et al., 2009). Stimulation frequency dependence of the activated area as well as fc between ROI pairs decreased unless the rate of the CRI was increased (Pawela et al., 2009). Additionally, the duration of previous isoflurane exposition affected ROI-ROI and whole brain fc as well as power of the signal fluctuations under a dexmedetomidine-CRI (Magnuson et al., 2014).

Nevertheless, one study also reported stable fc over time under isoflurane and under a medetomidine-CRI (Paasonen et al., 2016a).

Finally, urethane has a particular relation to time, as fc consistently displayed a cyclic behaviour (Wilson et al., 2011; Zhurakovskaya et al., 2016) which is in agreement with cyclic patterns observed in EEG.

Taken together, time-dependent effects were found in the majority of studies addressing the effect of timing of image acquisition on the BOLD results.

3.4.2 Mice

3.4.2.1 Effects of different states of anaesthesia on BOLD responses to peripheral stimulation

Five references compared BOLD responses to electrical paw stimulation under different states of anaesthesia.

In one study the activated area and the signal intensity change did not significantly differ between three rates of medetomidine infusion (0.1, 0.6 or 1.0 mg/kg/h ip starting 15 min after a bolus of 0.3 mg/kg ip) (Nasrallah et al., 2014c). In all groups focal activation in S1 was detected and time courses of the signal change were similar between groups. However, the time point of measurement was relevant: irrespective of the infusion rate, activation was only detected from 60 min after bolus on.

Schroeter et al. (2017) focused on the laterality of BOLD responses to hindpaw stimulation with 0.7 mA at 1.2% and 1.5% isoflurane in C57BL/6 mice and found at both concentrations almost identical signal amplitude in contra- and ipsilateral S1. The authors note that amplitude of signal change and interindividual variability differed across isoflurane levels, however, they do not specify the direction of those differences or provide any quantitative measure.

Two studies compared responses to hindpaw stimulation among isoflurane, medetomidine, propofol and urethane, and Schroeter et al. (2014) additionally compared two doses per anaesthetic. Schroeter et al. (2014) found distinct spatial activation patterns (displayed in activation maps) and distinct signal time courses. Number and size of activated clusters tended to be larger under isoflurane and propofol than under medetomidine and urethane. However, with all anaesthetics activations were widespread and no significant difference was observed between ipsi- and contralateral responses. Except for propofol, lower anaesthetic doses yielded larger signal amplitudes upon stimulation. In the case of urethane and medetomidine, the dose also affected the signal time course by decreasing onset times and eliminating the initial dip at the lower doses.

Using randomly spaced single pulse train instead of block stimulation, Schlegel et al. (2015) confirmed spatially specific activation maps for the four anaesthetics, which were independent from the model used to generate them. Again, signal time courses were anaesthetic-specific, but for each anaesthetic, time courses within cortical ROI were similar and signal amplitudes highest in contralateral S1HL. While isoflurane and urethane showed both an initial dip and post-stimulus undershoot, medetomidine and propofol-anaesthetised animals displayed “pronounced negative and positive signal transients”. In contrast to the other three groups, responses in thalamus were not reliably detected under urethane.

Shim et al. (2018) also observed bilateral responses to forepaw stimulation (1 mA) under isoflurane (1.0-1.1%), but unilateral responses under ketamine/xylazine (25 and 1.25 mg/kg ip, respectively). While under isoflurane a response was always observed, only 47 out of 58 ketamine/xylazine sessions showed a detectable response.

Taken together, bilateral responses to unilateral stimulation are consistently reported for isoflurane, urethane and propofol. Under medetomidine, however, both uni- and bilateral responses were reported. Finally, a single study observed unilateral responses under ketamine/xylazine.

3.4.2.2 Effects of different states of anaesthesia on BOLD responses to central stimulation

A single optogenetic study compared responses to light stimulation of S1 in awake animals with those obtained under 0.7% isoflurane (Desai et al., 2011). In awake animals 11 ROI were activated, but only 6 under isoflurane. 4 of the 5 designated “key ROI” (S1, contralateral S1, S2, M1 and CPu; criterium that detected in all awake animals) were also found in anaesthetised animals, but the signal change was significantly lower in all of them. The goodness of fit between observed BOLD responses and canonical haemodynamic response function was significantly lower under isoflurane, and the peak correlation coefficient was reached with significantly higher delay of onset. Pairwise ROI-ROI correlations following optical stimulation were also significantly lower under anaesthesia according to ANOVA. Interestingly, all correlations which were significantly reduced in post-hoc analysis were connections involving the striatum.

Overall, this study suggests that BOLD responses and stimulated fc are significantly reduced in anaesthetised animals.

3.4.2.3 Effects of different states of anaesthesia on resting state BOLD measurements

3.4.2.3.1 Medetomidine doses and timepoints

Five studies investigated the impact of doses and/or timepoints of imaging on rsfMRI under medetomidine.

Grandjean et al. (2014) found that interhemispheric connectivity was higher both for a seed in sensory cortex and a seed in dorsal striatum under the lower of two investigated doses of medetomidine (low: 0.05 mg/kg iv bolus followed by 0.1 mg/kg/h CRI; high: 0.1 mg/kg iv bolus followed by 0.2 mg/kg/h iv CRI). Furthermore, under the lower dose, interhemispheric connectivity was observed for two additional seeds in sensory cortex which did not show bilateral correlations under the higher dose. Re-analysis of the same dataset with a focus on local connectivity did not show significant differences in ReHo between the two doses, neither in voxelwise nor in ROI analysis (Wu et al., 2017). However, small differences in ReHo between the two doses had an influence, to which of the other anaesthetic protocols significant differences were found.

Shah et al. (2016) obtained rsfMRI 20 and 50 min after administration of a single medetomidine bolus (0.3 mg/kg sc, no CRI thereafter). At 50 min the ICA component which was most similar to the default mode network contained more regions than at 20 min. Seed-based analysis confirmed significantly lower connectivity of left cingulate cortex with retrosplenial cortex and hippocampal regions, i.e. the posterior parts of default mode network, at 20 min.

Nasrallah et al. (2014c) compared data acquired under three different rates of medetomidine-CRI (0.1, 0.6 and 1.0 mg/kg/h ip after 0.3 mg/kg ip bolus) and at two timepoints each (30 and 120 min after bolus). Under 0.1 mg/kg/h, interhemispheric connectivities between various ROI (S1, S2, visual cortex, CPu, thalamus, hippocampus) were generally high at both timepoints. At 30 min, no significant difference between 0.1 and 0.6 mg/kg/h was found, but at 120 min, fc between bilateral thalamic nuclei was significantly reduced. Under 1.0 mg/kg/h, interhemispheric fc was significantly reduced in most ROI, except CPu, at both timepoints. Within S1, the mean peak frequencies of the correlation did not significantly differ between infusion rates. Between the two resting state scans an electrical forepaw stimulation paradigm was applied. Interestingly, the latency and the amplitude of signal change in the stimulation scans was significantly correlated to the strength of interhemispheric S1 correlation: the slower and weaker the response to stimulation was, the weaker the bilateral S1 connectivity was.

Mechling et al. (2014) compared connectivity matrices of two consecutive scans acquired 30 and 45 min after the medetomidine bolus (0.3 mg/kg sc bolus, 0.6 mg/kg/h sc CRI) and found only in 1.4% of functional clusters a significant difference in connectivity strength. Those did not affect the number of functional clusters or their property of small worldness. Contrary to the findings of Nasrallah et al. (2014c), no significant change in the connectivity of thalamic nuclei was detected.

Taken together, higher doses of medetomidine were consistently reported to reduce fc between ROI. A single study could not extend these findings to local connectivity. Reported effects of the timing of image acquisition on resting state measures were inconsistent: While Shah et al. (2016) clearly observed an effect of time (20 versus

50 min) after a single bolus of medetomidine, Mechling et al. (2014) did not find a significant difference between 30 and 45 min after bolus under a CRI, and in the study of Nasrallah et al. (2014c), the effect of the timepoint of image acquisition was dose-dependent (only observed for the intermediate infusion rate).

3.4.2.3.2 Medetomidine/isoflurane timepoints

Shah et al. (2016) observed that raw T2* signal was weakest 20 min after administration of a single medetomidine bolus (0.3 mg/kg sc) on top of 1.5% isoflurane, but significant differences to the baseline (1.5% isoflurane) were present for 50 min after bolus.

Grandjean et al. (2017) acquired resting state scans 20 and 55 min after the medetomidine bolus (0.05 mg/kg iv) under combined medetomidine (0.1 mg/kg/h)/isoflurane (0.5%) anaesthesia. Static fc was higher in the second scan in several components (barrel field 1 and 2, limb, visual and cingulate/retrosplenial cortices, ventral hippocampus), but lower in others (dorsal and lateral striatal component). Nevertheless, the same atoms (“elementary building blocks of whole-brain dynamic connectivity representing specific dynamic functional states”) were identified with dictionary learning in both scans and only in 3 out of 20 atoms significant increases in atom fluctuation were observed at the second timepoint.

Taken together, raw signal intensity as well as stationary fc were reduced 20 min after a bolus of medetomidine on top of isoflurane compared to later measurements.

3.4.2.3.3 Medetomidine versus isoflurane versus medetomidine/isoflurane

Two studies based on the same dataset compared fc under multiple anaesthetic protocols (Grandjean et al., 2014; Wu et al., 2017).

Specifically for medetomidine (0.1 mg/kg iv bolus followed by 0.2 mg/kg/h iv CRI standard dose, 0.05 mg/kg iv bolus followed by 0.1 mg/kg/h alternative dose), isoflurane (1.0% standard, 1.5% alternative concentration) and the combination thereof (lower medetomidine dose plus isoflurane 0.5%), Grandjean et al. (2014) found that interhemispheric connectivity was prominent between cortical seeds, but absent in subcortical seeds under isoflurane, whereas strong subcortical but limited, dose-dependent (in one out of three sensory seeds under 0.1 mg/kg/h, in three out of three under 0.5 mg/kg/h) cortical interhemispheric fc was observed under medetomidine. Under medetomidine/isoflurane, significant bilateral correlations were present in both cortical and subcortical seeds. Interhemispheric connectivity in a sensory seed was significantly higher under isoflurane 1% than under medetomidine 0.1 mg/kg/h, under isoflurane 1.5% than under both medetomidine doses, under medetomidine/isoflurane than under both medetomidine doses and under 0.05 mg/kg/h than under 0.1 mg/kg/h medetomidine. For a seed in dorsal striatum, interhemispheric fc was comparable under medetomidine/isoflurane and medetomidine 0.05 mg/kg/h, and significantly higher under medetomidine/isoflurane than under isoflurane 1%, as well as under medetomidine 0.05 mg/kg/h than under medetomidine 0.1 mg/kg/h and both isoflurane doses. When results were compared with analysis without global signal regression, extended correlations with a sensory

seed were observed under isoflurane, while those under medetomidine were “clearly better confined”. Unregressed medetomidine/isoflurane data also showed confined correlations. Peaks of the respective frequency distributions were at 0.01, 0.15 and 0.2 Hz for isoflurane, medetomidine/isoflurane and medetomidine. Approximate entropy, a measure for stochastic behaviour of signal fluctuations over time, was significantly higher under medetomidine/isoflurane than under both medetomidine doses.

Re-analysis of the same dataset, with the exception of isoflurane 1.5%, by Wu et al. (2017) focused on local connectivity, measured by ReHo. When voxelwise ReHo maps were generated, generally high values were observed in cortical areas, under medetomidine and medetomidine/isoflurane additionally in CPu, and under isoflurane and medetomidine/isoflurane additionally in hippocampus. Comparison of voxelwise ReHo over the entire brain showed significantly higher ReHo in cortical areas under isoflurane and medetomidine/isoflurane than under the high dose of medetomidine, whereas in the CPu, ReHo was significantly lower under isoflurane than under both medetomidine concentrations. Analysis of ReHo within 6 ROI revealed no significant differences between anaesthetic protocols in hippocampus and insular cortex, plus for the three anaesthetics reported here in the thalamus, but significantly higher ReHo under medetomidine/isoflurane than under the high dose of medetomidine in cingulate cortex, and significantly higher ReHo under medetomidine/isoflurane as well as under isoflurane than under the high dose of medetomidine in sensory cortex (barrel field). In CPu, ReHo was significantly lower under isoflurane than under the high dose of medetomidine as well as under medetomidine/isoflurane.

Schroeter et al. (2017) focused on strain differences in interhemispheric connectivity between C57BL/6, acallosal I/LnJ and BALB/c mice. Isoflurane concentrations of 1.3 and 1.5% were used as well as a combination of 0.5% isoflurane and medetomidine (0.05 mg/kg iv bolus, 0.1 mg/kg/h iv CRI). The authors report that anaesthetic depth affected amplitude of signal fluctuations and interindividual variability (without specifying the direction or providing any quantitative information), but not “qualitative” aspects of rsfMRI, such as that interhemispheric connectivity was absent for most regions in acallosal animals.

Taken together, Grandjean et al. (2014) and Wu et al. (2017) found strong fc in cortical areas under isoflurane, in subcortical areas under medetomidine and strong fc in cortical as well as subcortical areas when medetomidine and isoflurane were combined. This pattern was observed for both interhemispheric and local connectivity. However, both articles also demonstrate that fc patterns and strength are sensitive to the dose of medetomidine. As Schroeter et al. (2017) did not specify the effects of the investigated protocols, integrating these findings with those of the other two articles is not possible.

3.4.2.3.4 Multiple comparisons

Three articles based on two datasets addressed comparisons between multiple anaesthetics. In addition to isoflurane, medetomidine and medetomidine/isoflurane, Grandjean et al. (2014) and Wu et al. (2017) also investigated urethane (1.5 g/kg ip standard dose, 1.2 g/kg ip alternative dose) and propofol (30 mg/kg iv bolus, followed by 120 and later 150 mg/kg/h iv standard dose; 45 mg/kg iv bolus, followed by 187 and later 225 mg/kg/h iv alternative dose). Under all protocols bilateral connectivity

for a seed in sensory cortex was found, but only under isoflurane and propofol bilateral correlations were observed for additional seeds in sensory cortex (Grandjean et al., 2014). Striatal interhemispheric connectivity was limited to medetomidine protocols, however, for a seed in the limbic system, interhemispheric connectivity was additionally observed under urethane. Neither interhemispheric connectivity for a seed in the cingulate cortex nor correlations between the thalamus and sensory seeds were observed under any of the anaesthetic protocols. Quantitatively, interhemispheric correlations in the cortical seed were of comparable magnitude (no significant difference) under both isoflurane concentrations, medetomidine/isoflurane and propofol. Cortical interhemispheric connectivity was significantly lower under urethane, and significantly higher under urethane than under the high dose of medetomidine. Urethane and the low dose of medetomidine produced similar cortical interhemispheric connectivity strengths. For the striatal seed, interhemispheric connectivity strength was significantly lower under propofol, urethane and isoflurane than under medetomidine/isoflurane or the low dose of medetomidine.

Wu et al. (2017) included only standard doses (except for medetomidine) for the analysis of local connectivity. Voxelwise analysis revealed generally high ReHo values in cortical areas under all anaesthetic protocols. In CPu, ReHo values were high under urethane, along with medetomidine. When voxelwise ReHo over the entire brain was compared between groups, significantly higher cortical ReHo was found under propofol than under the high dose of medetomidine, and under isoflurane and medetomidine/isoflurane than under urethane. In contrast, ReHo was significantly lower in CPu under isoflurane than under all medetomidine containing protocols and urethane. ReHo in the hypothalamus and thalamus was significantly lower under medetomidine/isoflurane and the high dose of medetomidine, than under urethane and propofol, respectively. In ROI analysis, ANOVA identified significant differences between anaesthetic protocols in 4 out of 6 ROI (no difference in insular cortex and hippocampus). In sensory cortex, local connectivity was significantly lower under urethane than under isoflurane and medetomidine/isoflurane. In CPu, propofol significantly reduced ReHo compared to all medetomidine containing protocols. Finally, local connectivity within the thalamus was significantly higher under propofol than under isoflurane, medetomidine/isoflurane and the high dose of medetomidine and also significantly higher under urethane than under the high dose of medetomidine.

Jonckers et al. (2014) compared isoflurane (1%), α-chloralose (120 mg/kg ip) and urethane (2.5 g/kg ip) anaesthesia with awake imaging for rsfMRI. Results for the comparison of awake versus anaesthetised are described in the next section. In the authors' judgment, ICA identified "overall the same regions" and components were partially overlapping. Qualitatively, the authors describe spatially less confined components under isoflurane than under α-chloralose and urethane. For ROI-analysis, seeds were placed in left M1, S1 and CPu and fc assessed as cluster size of correlated voxels, maximal t value (Tmax) of correlated voxels and correlation coefficient between bilateral homotopic regions. Cluster size was significantly higher in the right CPu under α-chloralose than under the other two anaesthetics. Tmax was in right S1 significantly higher under isoflurane and the other two agents, but in right CPu significantly higher under α-chloralose than under the respective other two agents. A significant difference in bilateral correlation coefficients was suggested by ANOVA for M1 and S1, but significant in post-hoc Bonferroni test only in S1, between

higher correlation coefficients under isoflurane and lower correlation coefficients under α-chloralose. Correlation coefficients in CPu were low in all groups. Taken together, significant differences between anaesthetics were observed in this study in one out of six, two out of six and one out of three possible locations for the outcome measures of cluster size, Tmax and bilateral correlation coefficients. Overall, in the authors' interpretation these findings demonstrate reduced cortical interhemispheric fc under isoflurane relative to urethane and α-chloralose.

In summary, the two datasets share only the comparison of isoflurane with urethane. Under isoflurane cortical interhemispheric connectivity was consistently higher than under urethane, whereas no significant difference was found in striatal interhemispheric connectivity (Grandjean et al., 2014; Jonckers et al., 2014). Local connectivity followed the same trend in cortex but was in CPu significantly higher under urethane than under isoflurane in a single report (Wu et al., 2017). Overall, similar trends were observed by Grandjean et al. (2014) and Wu et al. (2017) for interhemispheric and local connectivities, i.e. anaesthetic protocols under which higher interhemispheric connectivity was found relative to others tended to show higher local connectivity as well, however, these analyses were based on the same dataset.

3.4.2.3.5 Awake versus anaesthetised

Two references compared rsfMRI from awake mice with rsfMRI from anaesthetised mice.

Using ICA, Yoshida et al. (2016) observed uni- and bilateral cortical and limbic networks in awake habituated mice (daily restraining in a mock scanner for 2 h over 8 days) as well as in medetomidine-sedated animals (0.3 mg/kg sc bolus, 0.6 mg/kg/h sc CRI), but basal ganglia networks were only found in sedated animals. Depending on methods of analyses, connectivities (of individual voxels with independent components) were significantly higher in awake animals in retrosplenial cortex and hippocampus. Analysis of pairwise ROI-ROI correlations, however, revealed only a trend (i.e. not significant) for higher connectivities in awake animals.

Jonckers et al. (2014) analysed fc in awake animals (acclimatisation over 8 days with increasing duration of restraint) and under isoflurane (1%), α-chloralose (120 mg/kg ip) and urethane (2.5 g/kg ip) anaesthesia. ICA identified in the authors' judgement overall similar and partially overlapping components in all groups, but a CPu component was not found in awake animals in contrast to all anaesthetised groups. When seed were placed in left M1, S1 (barrel field) and CPu, significantly higher cluster sizes of correlated voxels in awake animals than in all anaesthetised groups were only observed for the seed in S1 (significant in both hemispheres). Maximal t values were significantly higher in awake than in urethane- and α-chloralose-anaesthetised animals in the left motor cortex for the seed in M1, in the right somatosensory cortex for the seed in S1, and significantly higher than in all three anaesthetised groups in left somatosensory cortex for the seed in S1. Cross-correlation coefficients of left and right somatosensory cortex, but not motor cortices and CPu, were significantly higher in awake than anaesthetised animals.

Taken together, in both studies some aspects of fc were stronger/higher in awake animals – but not in all regions and not in all outcome measures trends reached significance – while other aspects were similar in awake and anaesthetised animals.

3.4.2.3.6 Summary

In summary, higher doses of medetomidine were consistently reported to reduce fc between ROI but reported effects of the timing of image acquisition under medetomidine alone or on top of isoflurane on resting state measures were inconsistent.

Fc tended to be strong in cortical areas under isoflurane, in subcortical areas under medetomidine and strong in cortical as well as subcortical areas when medetomidine and isoflurane were combined.

Under isoflurane cortical interhemispheric connectivity was higher than or not significantly different from under urethane, and no significant difference was found in striatal interhemispheric connectivity. While cortical interhemispheric connectivity was lower under medetomidine than under urethane, ReHo was under both agents relatively high.

Finally, awake imaging yielded tendentially stronger fc than anaesthetised imaging.

3.4.2.4 Conclusion

Although limited data is available on peripheral stimulation, it appears that bilateral activation after unilateral stimulation is a common problem and was reported under most of the investigated anaesthetics.

In rsfMRI, anaesthetics tended to be characterized by regional differences in fc strength, with some anaesthetics enhancing rather cortical and others rather subcortical fc.

Overall, response to stimulation and fc were tendentially stronger in awake than anaesthetised animals. While significantly higher response to optogenetic stimulation and stronger fc were observed in awake animals in an optogenetic stimulation study (Desai et al., 2011), trends were less pronounced in resting state studies and fc was only in some aspects significantly stronger in awake than anaesthetised animals (Jonckers et al., 2014; Yoshida et al., 2016).

Dose-dependence, at least partial, was observed in all three resting state datasets and in two out of three stimulation datasets for four different anaesthetics.

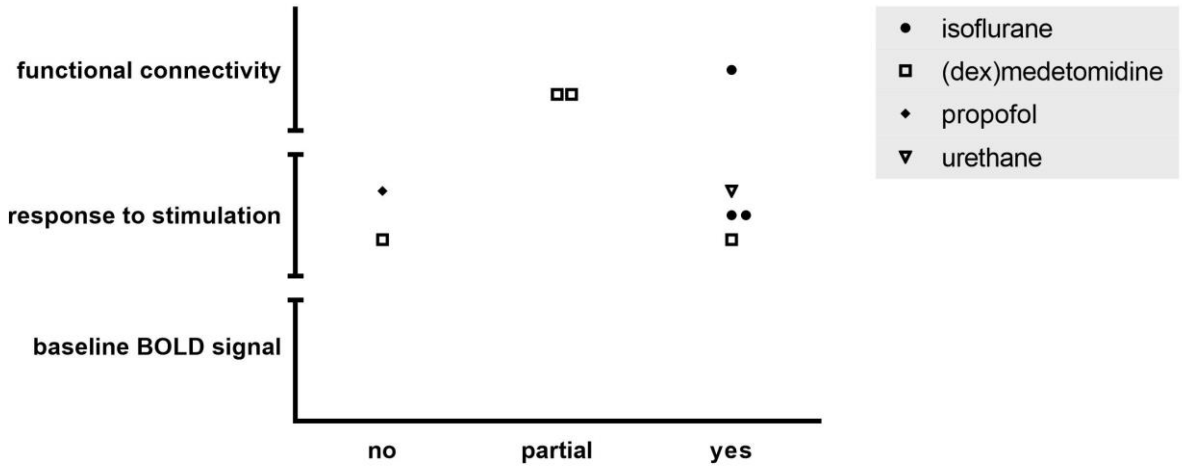

Figure 14. Dose dependence of responses to stimulation and fc in mice (no data available for baseline BOLD signal). No = no dose dependent effect was observed; partial = dose dependence was observed for some outcomes, analyses or animals, but not consistently; yes = a dose dependent effect was clearly observed. One point per dataset per anaesthetic. If original article and re-analysis disagreed, partial dose dependence was selected.

Those studies that investigated time dependence all used medetomidine alone or on top of isoflurane. They often, but not universally found an effect on at least some outcomes. Responses to electrical paw stimulation were only detectable from 60 min after bolus administration on, irrespective of the doses used (Nasrallah et al., 2014c). The same study found that at the intermediate infusion rate, thalamic interhemispheric fc was significantly lower than at the low infusion rate at 120 min, but not 30 min after bolus administration. For other regions and the high and low infusion rates however, no time-dependent differences were reported. This is in agreement with a study which did overall not find a significant difference in the number of functional clusters or their property of small worldness between two timepoints (30 and 45 min after bolus) (Mechling et al., 2014). In contrast, a third study reported that the number of regions contained in the component which is most similar to the default mode network was lower, and the connectivity of a seed in cingulate cortex with retrosplenial and hippocampal regions is reduced at 20 min compared to 50 min after bolus administration (Shah et al., 2016). The same study also assessed time dependence of raw T2* after administration of a medetomidine bolus on top of isoflurane and found lowest values and spatially most extended differences (relative to isoflurane) at 20 min after bolus administration. Grandjean et al. (2017) confirmed that stationary fc of several components was lower at 20 than at 55 min after bolus, but for other components the opposite was true. The number of functional units, the “atoms”, was meanwhile stable over time and only 3 out of 20 atoms showed higher fluctuations at the second time point of measurement.

Taken together, when time dependence was detected, fc did not uniformly increase with time elapsed since bolus administration.

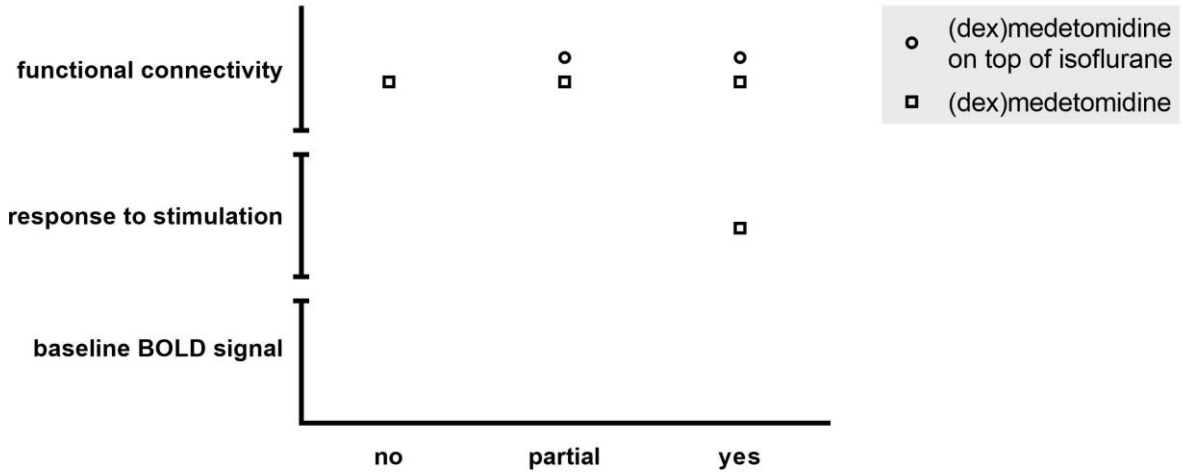

Figure 15. Time dependence of responses to stimulation and fc in mice (no data available for baseline BOLD signal). No = no time dependent effect was observed; partial = time dependence was observed for some outcomes, analyses or animals, but not consistently; yes = a time dependent effect was clearly observed. One point per dataset per anaesthetic. If original article and re-analysis disagreed, partial time dependence was selected.

4 Discussion

In this systematic review it was shown that p_aO_2, p_aCO_2 and arterial blood pressure affect BOLD signal across various experimental paradigms.

Responses to stimulation as well as fc are generally stronger in awake animals than in anaesthetised animals. When anaesthetised animals are imaged, anaesthetic dose- and time-dependent effects have to be expected. Strength and/or spatial extent of fc as well as responses to stimulation typically differ between different anaesthetics.

4.1 Physiological parameters under anaesthesia and the BOLD signal

Anaesthesia is a state of reversible unconsciousness which is accompanied by important changes in physiological parameters. Changes in p_aCO_2 and p_aO_2 commonly occur under anaesthesia and have the potential to bias BOLD fMRI results.

4.1.1 p_aCO_2

Direct depression of respiratory centres and peripheral muscle relaxation reduce minute alveolar ventilation in spontaneously breathing anaesthetised animals and lead to hypercapnia (Duke-Novakovski et al., 2016). Hypercapnia increases baseline signal while reducing response to stimulation. Studies either experimentally altered inspiratory gas compositions to test the effect of hypercapnia, adding 1 to 10 % of CO_2 to the baseline gas mix, or studied naturally occurring fluctuations. Significant changes were already observed with 1% CO_2 admixture, corresponding to an increase in p_aCO_2 of 5 mmHg (Nasrallah et al., 2015). Among two observational studies, one did not find that the magnitude of the BOLD response to (electrical paw) stimulation depended on p_aCO_2 (Sumiyoshi et al., 2012), but the other described abolished responses when p_aCO_2 exceeded 35 mmHg (Ramos-Cabrer et al., 2005). In human studies fluctuations in $EtCO_2$ as small as 1.1 mmHg are reported to correlate with fluctuations in global BOLD signal intensity (Wise et al., 2004). Local increases in pCO_2 trigger vasodilation to increase perfusion of areas with increased metabolism (Shockley and LaManna, 1988). Accordingly, increases in systemic p_aCO_2 increase global CBF. The relation between p_aCO_2 and CBF is almost linear between 20 and 80 mmHg (Reivich, 1964). When cerebral perfusion is increased without concomitant increase of $CMRO_2$, venous oxygenation increases and so does the baseline BOLD signal (Kim and Ogawa, 2012). Contrariwise, CBF response to stimulation is reduced when baseline CBF is already high (Cohen et al., 2002).

By which mechanisms and to which extent hypercapnia affects fc is less clear. A single study found increased interhemispheric S1FL connectivity and increased amplitude of signal fluctuations under one of several tested CO_2 concentrations, however, spatial extent of fc maps was unchanged and there was no correlation between p_aCO_2 and amplitude of signal fluctuations or between responses to stimulation and fc, so that the authors question the cardiovascular origin of the observed changes. In humans, hypercapnia was also shown to affect interhemispheric connectivity between homotopic regions (Marshall et al., 2015) and a recent publication claims that fc mapping is possible with data from cerebrovascular reactivity studies, when the effects of CO_2 are subtracted (Hou et al., 2019). Taken together, effects of hypercapnia – or even increases of p_aCO_2 within physiological ranges – on rsfMRI measures should be expected until proven otherwise. Throughout image acquisition, p_aCO_2 or at least endexspiratory CO_2 as a surrogate for arterial p_aCO_2 should therefore be monitored to avoid influences of variable p_aCO_2 on BOLD fMRI results.

Monitoring CO_2 levels is not only important in spontaneously breathing animals, but also in mechanically ventilated animals. Setting minute ventilation lower or higher than the actual requirement will over time lead to hyper- or hypocapnia. Although much less investigated than hypercapnia, hypocapnia induced by hyperventilation increased the amplitude of signal change and the activated area in an electrical forepaw stimulation study (Hsu et al., 1998), which is little surprising considering that hypocapnia provokes strong vasoconstriction and reduction of CBF (Severinghaus and Lassen, 1967). Monitoring of CO_2 allows ventilatory settings to be adjusted before significant changes in p_aCO_2 develop and thus helps to keep p_aCO_2 within a narrow range during the experiment.

4.1.2 p_aO_2

Hypoxaemia is another common complication of anaesthesia. The combination of reduced respiratory muscle activity (reducing the lung volume), atelectasis, ventilation-perfusion mismatch and hypercapnia results in reduced p_aO_2 (Lumb, 2019). Hypoxaemia decreases baseline signal and has variable effects on response to stimulation. With lower baseline arterial oxygenation, venous oxygenation decreases and so does BOLD signal intensity (Kim and Ogawa, 2012). Variable responses to stimulation may be partially explained by the fact that in one study, animals were breathing spontaneously and hyperventilating during hypoxic challenge (Sicard and Duong, 2005). The resulting hypocapnia led to vasoconstriction overruling hypoxic vasodilation. In the other study, animals were artificially ventilated – nevertheless, this study also reported small, insignificant decrease of CBF under hypoxia (Huang et al., 2013). Furthermore, SpO_2 was reduced to 75% and 81% in the first study (FiO_2 of 0.09 and 0.12) but only to 87% in the second (FiO_2 0.15). It is conceivable that in the latter study, arterial oxygenation was still high enough that the higher CBF response which was measured (compared to baseline) could account for the higher BOLD response.

Whatever the effects of different levels of hypoxaemia on BOLD responses are: p_aO_2 or SpO_2 should be monitored during fMRI and O_2 supplemented to prevent desaturation. FiO_2 should be high enough to prevent hypoxaemia, but low enough to prevent confounding effects of hyperoxia. Hyperoxia increases baseline signal, fc and potentially also response to stimulation (this was however not consistently found). The proposed mechanism is that elevated p_aO_2 reduces the amount of O_2 dissociating from haemoglobin, because O_2 dissolved in the plasma readily diffuses into surrounding tissues (Liu et al., 2019). Baseline gas mixtures in the included studies ranged from room air to 47% inspiratory oxygen. Most studies used 100% O_2 as hyperoxic condition, but one study found significantly increased baseline signal already at 40% O_2 (Baskerville et al., 2011). Based on current evidence, FiO_2 in the range of 0.25 to 0.40 appears reasonable for BOLD fMRI, but further research is needed to identify optimal concentrations.

Data presented here about the effects of p_aCO_2 and p_aO_2 alterations on BOLD result are almost exclusively from rats, as only one study investigated different gas compositions in mice. Results of that publication are however in line with rat data. An additional observational study in mice found a linear association between O_2 delivery, calculated as SpO_2 times CBV, and BOLD signal change during somatosensory stimulation.

That the described effects were observed under different anaesthetic protocols and generally in spontaneously breathing as well as ventilated animals shows that the effects are robust. Consequently, p_aCO_2 and p_aO_2 need to be measured, controlled and kept constant throughout the experiment in all BOLD fMRI experiments.

4.1.3 Arterial blood pressure

The third parameter for which relatively ample literature was found is arterial blood pressure. While arterial blood pressure increases are consistently reported to increase baseline BOLD signal and response to stimulation, decreases are inconsistently reported to decrease baseline BOLD signal and their effect on response to stimulation is controversial (from decreased over unchanged to enhanced responses).

Blood pressure increases were produced by noradrenaline boli or infusions of maximally 1 min in four out of five studies. Noradrenaline is generally assumed not to affect cerebral vasculature due to the blood brain barrier (Hardebo and Owman, 1980). Accordingly, MAP and thereby cerebral perfusion pressure should increase without affecting autoregulation. In principle, cerebral autoregulation should maintain CBF at constant levels for MAP of 50 to 140 mmHg by adjusting cerebral vascular resistance (Zaharchuk et al., 1999). To which degree autoregulation is preserved under anaesthesia depends however on the agent used. α-chloralose, which was used in four of the experimental hypertension studies, is reported to partially preserve autoregulation, although the upper limit may be reduced (Wang et al., 2010). For the

included studies it is often not obvious or difficult to reconstruct whether the induced blood pressure increases were within or beyond the autoregulatory limits reported by Zaharchuk et al. (1999). For example, baseline blood pressure is not reported in the studies by Qiao et al. (2007) and Tuor et al. (2002). It appears that while some BP increases exceeded 140 mmHg MAP (Wang et al., 2006), others were mainly within autoregulatory limits (Tuor et al., 2007). Irrespective of the blood pressure increases produced, the included studies generally ascribe the increase in baseline BOLD signal to hyperperfusion of the brain. In such a state of high perfusion pressure and rather constricted arterioles at baseline, increased responses to stimulation are plausible, as stimulation-induced dilation of arterioles will lead to a strong CBF response and a clear "surplus" of O_2 delivery.

Blood pressure decreases, on the other hand, were commonly induced by blood withdrawal. Approximately 1 to 10 ml/kg were withdrawn per step and at various rates. Additional variance was induced by the fact that one study (Kalisch et al., 2001) performed several steps of blood withdrawal and subsequent re-infusion. Although studies took care to avoid haemorrhagic shock, the higher volumes of 8-10 ml/kg may have activated compensatory mechanisms including release of vasoactive substances (Kalisch et al., 2001).

Across studies, both decreases within and below autoregulatory limits were usually observed within the same study. Except for Hempel et al. (1999), which states that a reduction of signal amplitude in response to stimulation was only observed when blood pressure fell below the autoregulatory limit, results usually refer to the total of blood pressure decreases. Nevertheless, some studies report more pronounced effects on baseline signal with larger blood pressure decreases (Wang et al., 2006). Compared to the blood pressure increase studies, more diverse anaesthetics were used in the blood pressure decrease studies. Besides α-chloralose, studies used isoflurane, halothane and in one case propofol. However, a significant correlation between blood pressure and BOLD signal was found under all three anaesthetics in one study investigating isoflurane, halothane and propofol, and with the limited number of included studies, no clear effect of anaesthesia on the detection of blood-pressure dependent effects was seen. Assuming that cerebral autoregulation was active, a decrease in cerebral perfusion pressure would increase CBV, which may, under certain circumstances, account for a higher total amount of deoxyhaemoglobin and accordingly lower signal intensity (Liu et al., 2019). When blood pressure was reduced below autoregulatory limits or in cases in which autoregulation may have been impaired, reduced cerebral perfusion readily explains the decrease in baseline BOLD signal: cerebral hypoperfusion increases O_2 extraction fraction and thus decreases venous oxygenation, which translates into lower BOLD signal intensity.

For response to stimulation, the situation is more complex: an abstract reports significantly enhanced responses to stimulation at 40 and 60 mmHg MAP induced with application of negative lower body pressure, but CBF was conserved at 60 mmHg and reduced at 40 mmHg (Herman et al., 2007), so the underlying mechanisms for the enhanced BOLD response must have been different at the two levels. In contrast, responses to stimulation were decreased after blood withdrawal to MAP values below autoregulatory limits (Hempel et al., 1999) or did not significantly

change (despite a trend towards lower responses) when trimetaphan camsilate was administered (Wang et al., 2006). The anaesthetic used can hardly explain those contradictory trends as all three experiments were performed under α-chloralose anaesthesia. Similarly, negative lower body pressure produces similar haemodynamic changes as haemorrhagic hypotension (Johnson et al., 2014) so that the methods used to provoke hypotension appear unlikely to have influenced the cerebral haemodynamic response to stimulation. Assuming a situation of cerebral hypoperfusion, one would expect that the CBF response to stimulation is reduced, first because arterioles are at baseline already rather dilated and second because cerebral perfusion pressure is low enough that an additional decrease in vascular resistance will only return a small increase in CBF. In such a situation, a smaller increase in additional O_2 delivery and thus a smaller BOLD signal increase would be expected. However, BOLD signal depends on CBF, CBV and $CMRO_2$ and the changes in CBV during hypotension are as controversial as the findings of stimulation studies (discussed by Zaharchuk et al. (1999)). Further studies would be required to characterise how systemic hypotension modulates BOLD responses to stimulation. Interestingly, mean CBF is maintained when MAP approaches the autoregulatory limit, but CBF fluctuations increase and thereby BOLD signal fluctuations increase (Kannurpatti et al., 2008).

Blood pressure changes can affect BOLD fMRI results in two situations:

First, hypotension, defined as MAP below 60 mmHg or systolic arterial blood pressure below 80 mmHg (Duke-Novakovski et al., 2016), is a common complication of anaesthesia. Second, stimuli applied during fMRI can provoke concomitant arterial blood pressure increase or, less commonly, decrease. Many substances tested in phMRI change arterial blood pressure for variable durations. Additionally, noxious or intense peripheral stimuli, such as application of irritating substances, can increase arterial blood pressure. For example, arterial blood pressure increases, and BOLD signal increases are significantly linearly correlated after formalin injection (Tuor et al., 2002). Especially abrupt blood pressure changes could override autoregulation for a short duration and introduce artefactual – positive or negative – activations. A study in rats did not find a significant correlation between the BOLD response to electrical paw stimulation and MAP (MAP ranging from 60 to 140 mmHg); however it is unknown whether paw stimulation elicited blood pressure changes (Sumiyoshi et al., 2012). In mice, stimulation-associated cardiovascular responses are suspected to account for bilateral responses to unilateral electrical paw stimulation (Schroeter et al., 2014; Schlegel et al., 2015; Schroeter et al., 2017). Cardiovascular responses were however characterised only by pulse oximetry (heart rate and pulse distension) and arterial blood pressure was not measured in those studies. Interestingly, cardiovascular changes were not detected when randomised single pulses were applied and activation of ipsilateral cortex was reduced, although not abolished. As bilateral responses to unilateral stimulation were even observed in acallosal mice, a cardiovascular or at least unspecific neural arousal response seem to best explain those unspecific activations.

Overall, despite some controversies about the underlying mechanisms and the direction of effects, arterial blood pressure changes have to be expected to modulate

the BOLD signal and may introduce artefactual activations or correlations when not detected and corrected for. Blood pressure should therefore be measured during fMRI. A single report suggests that correcting test-substance-induced hypotension in phMRI with a phenylephrine-CRI allowed detection of specific responses, which were otherwise obscured by arterial blood pressure correlated BOLD signal decreases. While this approach may be useful under specific circumstances, it should not be applied generically, as “overcorrection” of blood pressure also bears the risk to introduce artefacts.

4.1.4 General considerations

It is important to consider physiological parameters not as isolated entities: changes in one parameter will likely trigger changes in others. For example, spontaneously breathing animals exposed to hypercapnic or hypoxic inspiratory gas mixtures will increase minute ventilation in an attempt to normalise p_aCO_2 or p_aO_2, and at the same time also change partial pressures of the respective other gas. In other words, hypoxaemic spontaneously breathing animals become hypocapnic with time. Similarly, during apnoea or severe hypoventilation, p_aCO_2 and p_aO_2 in- and decrease concurrently. But not only respiratory aspects are interrelated. Mild hypercapnia or hypoxaemia increases arterial blood pressure and heart rate via sympathetic stimulation (Kuznetsova and Kulikov, 2014; Prabhakar, 2015), whereas severe hypoxaemia decreases arterial blood pressure (Kannurpatti and Biswal, 2004; Sicard and Duong, 2005). Furthermore, body temperature modulates both cardiovascular parameters and metabolism which in turn affects O_2 consumption and CO_2 production (Kurz et al., 1996).

This interdependence of physiological parameters may explain some of the variability of the results. Additionally, a range of anaesthetics was used across studies. Although those studies which compared effects of physiological parameter alterations on BOLD fMRI result found qualitatively similar effects, some quantitative differences were noted, which may also add variability to the results.

Finally, interventional studies more commonly reported an influence of physiological parameters on the BOLD signal than observational studies: 36 out of 39 interventional, but only 3 out of 6 observational studies found a significant effect. A possible explanation is that induced changes of physiological parameters were larger than naturally occurring changes. Alternatively, other physiological parameters may have been better controlled for in interventional studies, but when the percentage of studies which ventilated all animals is taken as an indicator for the control of physiological parameters, interventional and observational studies are in a similar range with 67% (26 of 39 studies) and 57% (4 of 7 studies), respectively.

4.2 States of anaesthesia and the BOLD signal

Comparison of anaesthetic protocols, or more precisely, different states of anaesthesia including awake state, revealed in rats as well as mice three general effects: differences between awake animals and anaesthetised animals, dose-dependence and time-dependence of results.

Results for dexmedetomidine and medetomidine were pooled in the present study as sedative and cardiovascular effects and pharmacokinetics of the two drugs are similar in rats (Savola and Virtanen, 1991) and mice (Burnside et al., 2013). Doses of medetomidine and dexmedetomidine are generally considered equivalent when dexmedetomidine is administered at 50% of the medetomidine dose (Burnside et al., 2013).

4.2.1 Awake versus anaesthetised

There was a clear trend for stronger and/or more extended fc and responses to stimulation in awake animals. Awake imaging was compared to imaging under isoflurane, medetomidine, propofol, α-chloralose or urethane. Irrespective of the anaesthetic used, fc was at least partially stronger in awake animals in rats as well as in mice. Responses to stimulation were more variable: one study each under medetomidine and α-chloralose found no significant difference to the awake state in rats (for mice, only a single stimulation study was available). In one case, the response to stimulation was even stronger under isoflurane: during severe hypoxaemia, BOLD signal decreased more in anaesthetised than in awake rats. Hypoxic stimulation differs from other types of stimulation by the fact that a decrease of BOLD signal intensity is expected as opposed to an increase in conventional stimulation paradigms such as somatosensory stimulation. This single study points at the possibility that when the response to a stimulus arises from a system being disturbed, higher responses may be observed in anaesthetised animals which have lower capacities to compensate.

Albeit overall stronger fc and responses to stimulation in awake animals, one has to keep in mind that the goal of fMRI is not to measure the highest signal intensities, correlation coefficients or spatial extents of networks or activations, but the most accurate. Some studies (e.g. Peeters et al. (2001)) concluded that although stronger, responses were also less specific in awake animals. In human fMRI it is a known problem that awake humans don’t exclusively think about the task they are given and that “unrestrained cognition” in the case of rsfMRI literally can go in any direction (Chou et al., 2017). Generally, such effects can be expected in awake rodents too. Specifically, stress experienced during fixation in a loud environment could be a major contributor to unspecific responses to stimulation or widespread, unspecific connectivity.

The circumstances under which animals underwent awake imaging varied between studies. Some studies (six datasets, six references) fixed anaesthetised rats on the animal cradle and acquired fMRI scans once the animals were considered to have recovered from anaesthesia. Waking up tightly restrained in an unfamiliar, loud environment is a stressful event for a rodent. Some studies report respiratory rates (Brevard et al., 2003) and heart rates, MAP and arterial blood gases (Duong, 2007), within normal ranges for awake animals but at the same time, one of them (Duong, 2007) noted that struggling movements “markedly decreased after the first hour”, indicating that animals were stressed despite apparently normal respiratory rates. Two studies (Peeters et al., 2001; Airaksinen et al., 2012) kept the animals paralysed after discontinuing inhalant anaesthesia. In one of those studies (Airaksinen et al., 2012), status epilepticus was induced during which animals were considered unconscious, but prior to this, baseline images were acquired in paralysed awake animals. Awareness during muscle paralysis is known to be highly stressful for humans and can lead to post-traumatic stress disorder (Sandin et al., 2000; Lennmarken et al., 2002). Discontinuing anaesthesia in paralysed animals is thus an inacceptable practice to prevent motion artefacts in awake imaging. One of the studies in question observed less specific and more variable responses to stimulation, which the authors judged as outweighing the larger signal changes. When it comes to stress, the aims of science and animal welfare are congruent: reducing stress improves data quality and animal welfare.

An approach to reduce stress during awake imaging is to use acclimation protocols. Most acclimation protocols encountered in the included studies are modifications of the protocol presented by King et al. (2005). It involves fixing anaesthetised rodents in the position required for imaging and leaving the animals restrained for a defined timespan once they regained consciousness. The procedure is repeated daily over several days. “Mock scanners” with or without recordings of MRI sounds as well as the actual scanners can be used. To reduce discomfort, lidocaine paste is applied on pressure points. In the original protocol, respiratory rate was significantly lower from day three and heart rate and corticosterone levels were significantly lower from day 5 on compared to the first training session. In contrast to the original protocol, which used ketamine/medetomidine for initial anaesthesia, all eight datasets included in this review used isoflurane. Among those eight datasets a cluster of three from the same group (at Penn State University), from which eight publications arose, used a version in which the duration of restraint was increased in steps of 15 min until the target duration was reached. Stress levels were not addressed in those publications. A publication in mice, using a very similar modification of the protocol, found that respiratory rate was significantly higher than in freely behaving mice and did not decrease throughout trainings and concluded that mere repetition of the exposure failed to decrease stress in mice (Jonckers et al., 2014).

Approaches to refine awake imaging include fixing animals via implanted head posts instead of stereotaxic ear- and bite bars and to use noise-reducing earplugs (Tsurugizawa et al., 2010). Similar to King et al. (2005), Tsurugizawa et al. (2010) found a decrease of respiratory and heart rate on days four and five of training, respectively. Beyond repeated exposure, a study in mice used chocolate sprinkles

during and at the end of the restraining period as positive reinforcement (Desai et al., 2011), however, they did not report signs of stress over the course of the training.

Even if stress decreases over the course of the acclimation protocol – which appears to be the case in rats, but not in mice, concerns remain with the use of anaesthesia for initial immobilisation as well as with repeated exposure to stress: first, effects of anaesthesia on brain functions might last longer than unconsciousness. Humans experience increased cognitive failures up to 3 days after regaining consciousness (Tzabar et al., 1996). Similarly, findings of Magnuson et al. (2014) indicate that effects of isoflurane exposure last longer than residual isoflurane concentrations can be expected to be present in the body (Sommers et al., 2009). For set-up in awake imaging, animals are typically anaesthetised much shorter than in the study of Sommers et al. (2009), only for a few minutes, and waiting periods of around 30 min before imaging is started are common, but those rely on subjective estimations rather than experimental data. Carry-over effects from anaesthesia on fMRI results can therefore not be excluded. Second, anaesthesia per se induces a physiologic stress response (Altholtz et al., 2006). Third, isoflurane and sevoflurane both become more aversive with repeated exposure in rats and mice (Wong et al., 2013; Moody and Weary, 2014; Bertolus et al., 2015; Hohlbaum et al., 2017). Inhalant anaesthesia for immobilisation is therefore a persistent source of aversion and (at minimum physiological) stress in acclimation protocols. Finally, although stress during restraint appears to decrease in rats over the course of the acclimation protocol, it is at least initially present and repeated exposure to stress may itself alter outcomes. Henckens et al. (2015) found increased connectivity in “somatosensory, visual and default mode networks” in rats which were immobilised 2 h per day on 10 consecutive days. On the other hand, Liang et al. (2014) did not find a significant difference in the time spent on open arms of an elevated plus maze, a measure for anxiety, between animals acclimated with their protocol and control animals. However, behaviour in the elevated plus maze was assessed 12 days after the last acclimation session (Liang et al., 2014). While the described acclimation protocols may improve image quality by reducing motion artefacts, cardiovascular arousal and eventually stress during imaging, repeated stress during acclimation is a potential confounder.

As any awake imaging which uses anaesthesia to position the animals in the scanner faces the risk of carry-over effects from anaesthesia on fMRI results, two studies acclimated animals to restraint without using anaesthesia. One study simply omitted anaesthesia and restrained awake mice for 2 h per day on 8 consecutive days in a mock scanner. Heart rate significantly decreased from day 7 of training on and the weight of faecal pellets was not significantly higher than in the control group from day 6 on. Notably, muscle activity was already on day two significantly lower than on day 1, suggesting that decreased struggling indicates learning effects rather than a reduction in perceived stress. Stress was not as severe that animals stopped gaining weight during the acclimation period. In the second study (Chang et al., 2016), rats were stepwise (30 min per day, 8 to 10 days within a 14-day period) trained to enter a snuggle sack and stay in there, to tolerate fixation by headposts (set-up so that they could initially free themselves), to tolerate receiving air puffs and to MRI noise. There was no significant difference between the awake and anaesthetised scan in

respiratory rate, and respiratory rate in the awake condition was with 74 +/- 6 breaths per minute lower than in a range of other awake imaging studies (King et al., 2005). Corticosterone levels did not significantly differ during training from baseline, which indicates that the animals were not chronically stressed by the training phase. However, despite training, corticosterone levels were significantly higher immediately after awake imaging than at baseline or during training, indicating that acclimation could not completely eliminate the stress of the actual imaging session.

Despite at first view promising levels of responses and fc, common awake imaging protocols risk to yield unspecific results and pose constraint on the animals. In order to enhance translation of findings in animal studies to humans, animals need to be specifically trained to voluntarily undergo awake imaging.

4.2.2 Dose effects

An effect of anaesthetic doses on fMRI results was commonly observed, but not for all anaesthetics at the same frequency and reported effects were not necessarily monotonic.

While dose-dependence was clearly observed for isoflurane, it was less pronounced for medetomidine (see figures 13 and 14). Dose-dependent rsfMRI results were observed under propofol, but responses to stimulation were not dose-dependent in a single study in mice. For halothane, urethane and S-ketamine, only single reports were found.

Among isoflurane studies, the investigated concentrations ranged from 0.5 to 3.0%, corresponding to 0.35 to 2.1 x MAC (Flecknell, 2015). rsfMRI studies in rats under isoflurane indicate monotonic decrease of fc strength between multiple “units” with increasing isoflurane concentrations and non-monotonic dose-dependence of interhemispheric fc strength, spatial extent of fc and signal fluctuations. The trends for interhemispheric fc strength and spatial extent of connectivities would be compatible with an inversed U-shape, whereas an overall decreasing trend with a local minimum close to MAC was observed for signal fluctuations. Comparison with findings in mice is not possible, as the only dataset available did not report the direction of differences in signal fluctuations between isoflurane levels. Regarding baseline BOLD signal, studies in rats consistently report an increase in baseline BOLD signal when isoflurane concentration is increased from 1.5 to 2.5% (Tsurugizawa et al., 2016; Abe et al., 2017), but disagree whether the trend continues in higher ranges (1.8 to 3.5%, (Gong et al., 2014) or is reversed (Tsurugizawa et al., 2016). The amplitude of responses to electrical peripheral stimulation decreased in both species when the concentration of isoflurane was increased from 1 to 3% (Nasrallah et al., 2014a) and from 1.0 to 1.5% (Schroeter et al., 2014).

For medetomidine, data about rsfMRI and electrical paw stimulation in rats originated from the same three studies investigating both modalities. While a limited dose-

dependent effect on response to stimulation was observed only after more than 2 h of CRI, interhemispheric fc strength of S1 was consistently reduced with higher infusion rates within the first 2 h and correlation coefficients between multiple ROI showed dose-dependent development over time thereafter. In mice a similar trend for clearer dose-dependence of rsfMRI than responses to stimulation was found. While two studies consistently report reduced interhemispheric fc between sensory, striatal and thalamic seeds with higher infusion rates, conflicting results were reported as to whether amplitudes of signal change are higher under lower infusion rates (one study per position). rsfMRI studies in mice did however not agree on the specific infusion rates for which a difference can be observed.

Generally, no significant changes in amplitudes of signal fluctuation were reported in rats, but significant reductions were observed in specific frequency bands in specific areas. Interestingly, in CPu neither interhemispheric fc nor amplitudes of signal fluctuation were significantly reduced with higher infusion rates. In mice one study found preserved interhemispheric fc of the CPu under an even higher infusion rate (1.0 mg/kg/h ip) than in rats (0.1-0.3 mg/kg/h ip or iv). As the CPu has a lower α_2-receptor density than the cortex (Unnerstall et al., 1984), it is not surprising that sensitivity of that area to medetomidine doses is less pronounced than in other areas.

Dose-dependence of the BOLD signal arises from the interaction of dose-dependent agent-specific neural and cardiovascular effects.

Generally, neural activity is reduced with deeper levels of anaesthesia, as evident in EEG and local electrophysiological recordings (Alkire et al., 2008). Under isoflurane EEG shows a burst suppression pattern around 1.8-2% isoflurane (Masamoto et al., 2009; Liu et al., 2011; Nasrallah et al., 2014a), and an almost flat line with minimal EEG power across all frequencies at 3% (Nasrallah et al., 2014a), indicating a global suppression of neural activity. Somatosensory evoked potentials are markedly decreased when isoflurane concentrations are increased and almost absent at 3% isoflurane (Nasrallah et al., 2014a) and evoked field potentials responses to block stimulation decrease with increasing isoflurane concentrations (Masamoto et al., 2009). The picture is somewhat more complex for (dex)medetomidine: generally, (dex)medetomidine induces a NREM sleep-like pattern in EEG (Huupponen et al., 2008) and frequencies decrease while amplitudes increase with higher medetomidine plasma concentrations (Bol et al., 1999). Square root power of low frequencies in EEG relative to dexmedetomidine plasma concentrations follows a sigmoid curve (Bol et al., 1997; Bol et al., 1999; Bol et al., 2000), meaning that a plateau of EEG effects is reached above a plasma concentration of approximately 4 ng/ml. As a 10 min infusion of dexmedetomidine at the rate of 180 ug/kg/h – without preceding bolus, as typically used in imaging studies – resulted in peak plasma levels of 16.7 +/- 1.3 ng/ml (Bol et al., 1997), the lower doses of 0.1 to 0.3 mg/kg/h medetomidine in Nasrallah et al. (2014a) may have reached the according plasma concentrations for medetomidine, explaining why no significant difference in EEG power was detected. Together with the observation that somatosensory evoked potentials did not significantly differ between 0.1 and 0.3 mg/kg/h infusion rates, and the observation

that above a certain threshold dose only the duration of sedation, but not the intensity increases with higher bolus doses in rats (Doze et al., 1989) and dogs (Vainio et al., 1989), it appears possible that a plateau of neural depression is reached with commonly used doses. However, both clinically appreciable sedation scores and power of the EEG provide rather undifferentiated assessments of neural activity. For example, while there was no difference in EEG total power, Nasrallah et al. (2014a) found a significant reduction of interhemispheric coherence of the EEG in the γ-band under higher infusion rates corresponding to reduced interhemispheric connectivity. Conceptually, anaesthesia-induced neural depression is more than the “sum of individual neurons’ reduced activity”. While the few-lead EEG measurements used in the cited studies may detect a reduction in information, the integration of this information cannot be assessed (Alkire et al., 2008).

Nevertheless, for most anaesthetics, the neural activity triggering changes in BOLD signal intensity should be reduced with higher doses of the anaesthetic.

As the BOLD signal is an indirect measure of neural activity, dose-dependent cardiovascular effects interact with dose-dependent neural effects.

Isoflurane causes dose-dependent vasodilation, thereby increasing CBF, and dose-dependent MAP reductions (Lenz et al., 1998; Ohata et al., 1999; Constantinides et al., 2011). The increase of baseline BOLD signal intensity with higher concentrations can be explained by the combination of increased CBF and reduced O_2 consumption due to depression of neural metabolism, resulting in a lower O_2 extraction when higher doses of isoflurane are used (Ohata et al., 1999; Gong et al., 2014). Dose-dependent cerebral vasodilation may further contribute to the reduced BOLD responses to stimulation (Ohata et al., 1999): Maximal dilation of vessels in response to stimulation is limited when vessels are already dilated at baseline. Consequently, local increases in blood flow and thus increases in oxygenated haemoglobin are limited (Gao et al., 2017). Higher fc values and more extended fc maps at higher isoflurane concentrations could be explained by lower MAP at higher concentrations. Mice have significantly lower MAP at 2 than at 1% isoflurane (Constantinides et al., 2011) and a decrease of MAP from 110 +/- 10 to 68 +/- 7 mmHg was shown to increase CBF fluctuations, which enhanced spontaneous BOLD signal fluctuations (Kannurpatti et al., 2008), thereby increasing the likelihood that some fluctuations reach significance. The suspected inverse U-shape or at least non-monotonic trends observed for some rsfMRI outcomes could be explained by two mechanisms. First, the effect of increased CBF may be offset by reduced neural activity at some point. Second, very high isoflurane concentrations may reduce MAP below autoregulatory limits, so that despite cerebral vasodilation CBF cannot be maintained.

In contrast to isoflurane, (dex)medetomidine is a vasoconstrictor (Ganjoo et al., 1998) and its effect on cerebral vasculature is not dose dependent (Ohata et al., 1999). That responses to stimulation showed in most studies no dose-dependence is not surprising when neither neural responses to stimulation nor cerebral vessel diameter are dose-dependent. Evoked responses in primary sensory cortices are furthermore in general relatively insensitive to anaesthetic depth (Alkire et al., 2008). There is evidence from electrophysiological recordings as well as from rsfMRI that association

networks are more sensitive to anaesthetic depth than sensory networks (Sellers et al., 2013; Liang et al., 2015a). Indeed, rsfMRI results were also under (dex)medetomidine in most of studies at least partially dose dependent. If cerebral haemodynamics are less affected by different doses of (dex)medetomidine than by different concentrations of isoflurane, those partial dose-dependences in rsfMRI may reflect altered neural activity. That the two rsfMRI studies in mice did not agree on the specific infusion rates for which a difference can be observed, can be explained by several factors. First, Nasrallah et al. (2014c) administered the same initial bolus of 0.3 mg/kg to all groups before continuing with 0.1, 0.6 or 1.0 mg/kg/h infusion rates, whereas Grandjean et al. (2014) gave double the initial bolus in the higher infusion rate group (0.1 mg/kg followed by 0.2 mg/kg/h versus 0.05 mg/kg followed by 0.1 mg/kg/h). A single sc bolus in rats had an elimination half-life time of 1.6 h and peak concentration in the brain was reached after 15 to 20 min (Salonen, 1989). After iv administration an average plasma/effect-site equilibration half-life of 8.6 min is reported for dexmedetomidine (as assessed by EEG in Bol et al. (1997)). Consequently, a single bolus, even if administered ip or iv, should still have an effect at 30 min post bolus, when the first measurements were acquired in both studies (Grandjean et al., 2014). Additionally, isoflurane was discontinued in Nasrallah et al. (2014c) only 15 min before the first scan, so there may have been a residual effect, and the CRI was started only 15 min before the first scan, a time span after which steady state plasma levels are not yet established. The cumulative dose of medetomidine in Nasrallah et al. (2014c) was thus 0.325 mg/kg in the low rate group and 0.45 mg/kg in the intermediate rate group. The relative difference between groups was accordingly larger at the later timepoint (120 min), where bolus effects should have vanished and differences been dominated by the infusion rate. Second, methods of analysis and outcome measures differed between the two studies (Nasrallah et al. (2014c) seed based, Grandjean et al. (2014) ICA based).

4.2.3 Time effects

While dose matters, time matters too. In rats and mice, responses to stimulation were reduced after a bolus of (dex)medetomidine (Brynildsen et al., 2017) or α-chloralose (Austin et al., 2005), or abolished for 60 to 80 min (Weber et al., 2006; Nasrallah et al., 2014c; Gsell et al., n.d.). Likewise, fc strength increased or connectivities became spatially more extended over time after administration of a bolus of dexmedetomidine or α-chloralose in rats (Bettinardi et al., 2015; Paasonen et al., 2016a; Brynildsen et al., 2017). In mice, results were less consistent for time-dependent effects of (dex)medetomidine CRI, alone or on top of isoflurane. While in some aspects of fc an increase was observed, others were unchanged or specific connections weakened (Grandjean et al., 2017). Changes of fc over time can occur in both directions concurrently, as observed for a subanaesthetic dose of ketamine on top of isoflurane (Gass et al., 2014). Finally, complex patterns of changes and interactions with doses were observed for prolonged medetomidine infusions (Pawela et al., 2009).

When it comes to time dependence of fMRI results, two key factors must be considered: first, reaching constant concentrations at the effect site, i.e. in the brain, is not trivial with injectable anaesthetic agents. Second, even if a constant exposure is maintained, anaesthesia targets a dynamic system: neurons and networks can adapt over time so that a constant exposure will not necessarily result in constant effects. Moreover, changes in the system's state can outlast the duration of exposure.

After a single bolus, plasma concentrations as well as concentrations at the effect site (i.e. in the brain) typically reach a peak before declining again. Accordingly, anaesthesia is deepest some time after the bolus – depending on route of administration, distribution etc of the agent – and wears off thereafter (Grimm, 2015). Transient abolishment or reduction of responses to stimulation after a bolus may thus mirror the time-course of concentrations at effect site. To prolong the duration of appropriate depth of anaesthesia for imaging, many studies start CRIs after the initial bolus (e.g. when using (dex)medetomidine, propofol or sometimes α-chloralose) or repeat boli at a certain interval (especially with α-chloralose). With repeated bolus administration, concentrations will rather fluctuate within a certain range than being constant (Flecknell, 2015). But constant rates of administration don't guarantee constant concentrations of the anaesthetic in the brain either, because pharmacokinetics of many agents are best described by a multi-compartment model (Flecknell, 2015).

For medetomidine, peak plasma concentrations are reached 10 min after sc administration of a bolus of 80 ug/kg in rats. Maximal concentrations in the brain are five times higher than in plasma and reach the peak 15-20 min after bolus (Salonen, 1989). Elimination half-life time is 1.6 h after sc administration and the elimination rate in the brain is similar to plasma, just somewhat delayed (Salonen, 1989). Short iv infusions of a constant rate (10 min) or stepped rates (50 min, 10 min per step) of dexmedetomidine result in mean plasma/effect site equilibration time of 8.6 min (Bol et al., 1997). Measurements of plasma concentrations during a prolonged CRI in rats are however not available. In ponies and dogs, constant plasma levels and constant levels of sedation are reached with CRIs after an initial bolus (Bettschart-Wolfensberger et al., 1999; Lamont et al., 2012).

Although α-chloralose has been used for decades in laboratory animal anaesthesia, details on its pharmacokinetics in rodents are not available. In the literature, α-chloralose is commonly reported to produce light anaesthesia for 8-10 h (Flecknell, 2015). Most studies included in this review either re-dosed animals after a certain time or used a CRI. Doses and intervals were however highly variable and there appeared to be no association with the route of administration (ip or iv): initial doses ranged from 25 to 120 mg/kg (one study each, 40-80 mg/kg in rest of studies), top-up doses from 20 to 40 mg/kg, intervals from 30 to 120 min (most studies 45 or 60 min) and infusion rates from 10 to 50 mg/kg/h. Plasma concentrations over time were not investigated in those studies, and are to the best of our knowledge not published for any of those protocols.

As discussed for dose-dependence of the results, variations in plasma concentration over time will affect both the neural (via concentrations in the brain) and cardiovascular basis of the BOLD signal.

In contrast to injection anaesthesia, concentrations at the effect site can be kept within a narrow range when using modern inhalant anaesthetics like isoflurane or sevoflurane which are almost not metabolised (0.17% of isoflurane (Eger, 1984) and 1-5% of sevoflurane (Patel and Goa, 1996)). Once an equilibrium between partial pressure of the inhalant anaesthetic in the alveolus and the blood and the brain has been established, partial pressure in the brain follows alveolar partial pressure. In intubated animals, measurement of end-tidal inhalant concentrations allows non-invasive, constant breath by breath real-time monitoring of alveolar concentrations of the inhalant. In the non-rebreathing systems which are typically used for rodents, where the inhalant is delivered with a high fresh gas flow, any change in vaporizer setting will rapidly change partial pressure of the inhalant agent and establish a new equilibrium (Grimm, 2015). Hence, inhalants have the double advantage that constant exposure is easy to provide and easy to monitor.

To achieve constant levels of anaesthesia, constant concentrations of the agent at the effect site are however not enough. Tolerance to certain drugs develops with repeated or prolonged exposure, either by enhanced drug metabolism and/or distribution, or by reduced neuronal sensitivity (Tripathi, 2013). With repeated ketamine administration for example, hepatic enzymes are induced and sleeping times decrease in rats (Livingston and Waterman, 1978; Albrecht et al., 2014). Propofol on the other hand showed signs of pharmacodynamic tolerance in EEG measurements in rats within 90 min of infusion (Ihmsen et al., 2005). Tolerance was not studied in any of the included references investigating ketamine or propofol, but Pawela et al. (2008) found that rats under a constant rate of medetomidine infusion woke up after 3.5 to 4 h, independent of the rate (0.1 or 0.3 mg/kg/h iv). Increasing the rate of infusion 2.5 h after bolus increased the duration of sedation to 6 h (Pawela et al., 2008), which the authors interpreted as a strong indicator for the development of tolerance. Unfortunately, plasma concentrations were not measured in that study, so that differentiation between pharmacokinetic and -dynamic mechanisms was not possible.

In conclusion, optimal timeframes for image acquisition under (dex)medetomidine or any other injectable anaesthesia protocol have to be determined based on pharmacokinetic and -dynamic data for the respective agents in the target species, to ensure that anaesthesia approaches a steady state during the whole imaging period. As tolerance does not occur with inhalants (Stratmann et al., 2009), steady state anaesthesia is readily achieved with isoflurane or sevoflurane anaesthesia. However, as the brain is a dynamic system, even if a constant level of anaesthesia is provided, this disturbance of the system may induce changes in the system which outlast the duration of exposure. One study reports that the duration of previous isoflurane exposure affected rsfMRI outcomes in rats under identical dexmedetomidine CRIs beyond the washout period of isoflurane (Magnuson et al., 2014). As many studies use isoflurane for induction and preparation before switching to (dex)medetomidine or other injectable anaesthesia protocols, exposure to isoflurane prior to imaging has

to be standardised in all animals and reported to allow comparison of results from different studies. Further research is needed to confirm and characterise the impact of duration of exposure on subsequent measurements. Beyond those practical implications, the findings of Magnuson et al. (2014) raise the possibility of time-dependent effects during prolonged imaging despite (apparently) constant levels of anaesthesia.

4.2.4 Comparisons between specific anaesthetics

Historically, α-chloralose was a standard anaesthetic in neuroscience because it preserves functional-metabolic coupling (Ueki et al., 1992), and it was commonly used in the first 15 years of BOLD fMRI. As rsfMRI of rodents became popular only in the mid-2000s, α-chloralose is in this review overrepresented in stimulation studies. When comparing across all stimulation paradigms for rats, there was a trend for higher signal changes and/or area of activation, including activations outside the primarily targeted region, under α-chloralose than isoflurane. Optimal frequencies for electrical paw stimulation appeared to differ between α-chloralose and medetomidine or urethane and potentially pentobarbital. Responses to stimulation were more localised and specific under α-chloralose than under halothane, but only for the first 1-2 h after bolus, which highlights the impact of doses and timepoints on the performance of one anaesthetic relative to another. Only one study investigated α-chloralose in mice and found that cortical interhemispheric connectivity was lower under α-chloralose than isoflurane. Despite overall robust responses to stimulation, α-chloralose has been replaced over the past years due to its major disadvantages. First of all, due to long, excitatory recovery phases, its use should be limited to terminal studies (Flecknell, 2015), which precludes longitudinal fMRI studies. Additionally, ip administration causes local inflammation and, in some species, adynamic ileus (Silverman and Muir III, 1993). Although de Celis Alonso et al. (2011) claim that α-chloralose can be used for longitudinal studies when administered iv, this practice has to be questioned: 2 out of 18 animals died after an α-chloralose anaesthesia, recovery times were around 2-3 h, and in contrast to isoflurane anaesthesia, tail flick responses were still delayed and rotarod performances reduced 24 h after α-chloralose anaesthesia and food intake did not normalise within 48 h, indicating an overall considerable burden for the animals. But even if α-chloralose is to be used for terminal studies, the following disadvantages should be considered: Due to slow onset of action (15 min after iv administration) and excitatory induction phases, α-chloralose is generally not considered a suitable anaesthesia induction agent (Flecknell, 2015; Zahner and Arras, 2016). Consequently, anaesthesia has to be induced with another agent, which introduces the risk of carry-over effects. Pharmacokinetics of α-chloralose are not well known and doses/protocols vary widely between groups. Furthermore, as α-chloralose is not registered as a medical product, it has to be prepared in the laboratory (based on pure α-chloralose or sometimes extracted from chloralose-based rodenticides), so that the quality of the end product is often unknown (Zahner and Arras, 2016).

Common alternatives to α-chloralose are isoflurane and medetomidine. Both yielded satisfactory results in stimulation studies. They have – in rats – however not been directly compared with each other for peripheral stimulation studies. Responses to deep brain stimulation were in a single report more reproducible in their signal amplitude, area of activation and localization of activations under dexmedetomidine than under isoflurane. In phMRI, results for the comparison of isoflurane and medetomidine were inconsistent, however this is to be expected because interference of anaesthesia with phMRI responses will inevitably depend on the tested substance.

In mice, BOLD fMRI was established later than in rats and accordingly, studies used from the beginning on more often isoflurane and medetomidine (than “early” rat stimulation studies). Stimulation studies in mice face the major problem of bilateral activations despite unilateral somatosensory stimulation, which is contrary to what is expected based on electrophysiological studies and fMRI studies in other species, and therefore generally interpreted as artefacts from (cardiovascular) arousal. Bilateral activations were observed under isoflurane, urethane and propofol. Under medetomidine, responses were unilateral in one study but bilateral in another. A recent publication obtained unilateral responses with a ketamine/xylazine protocol (Shim et al., 2018). As animals were markedly bradycardic throughout the study, findings could be explained by a suppressed cardiovascular response to stimulation. Indeed, heart rate did not increase upon stimulation, whereas Schroeter et al. (2014) found an increase in heart rate in animals under medetomidine. On the other hand, electrical stimulation is unpleasant even when not painful. Both ketamine and xylazine have strong analgesic properties and could thus reduce the response to such an unpleasant stimulus. Further studies are needed to confirm the results and eventually establish optimal doses and time points for imaging. As ketamine/dexmedetomidine is locally less irritating at ip administration than ketamine/xylazine, but causes more pronounced cardiorespiratory depression at comparable levels of anaesthesia (Wellington et al., 2013), the optimal α_2-agonist to combine ketamine with may also be discussed.

Interestingly, adding a dexmedetomidine-CRI (of 0.3 mg/kg/h) to isoflurane close to MAC (1.3%) increased the activated area as well as the signal amplitude relative to the baseline under isoflurane in rats (Nasrallah et al., 2014b). This combination was not tested in any of the included studies for stimulation in mice.

For resting state however, the combination of a low dose of isoflurane and medetomidine appeared to combine the strengths of both agents in mice. While isoflurane was associated with stronger cortical and medetomidine with stronger subcortical fc, the combination resulted in strong cortical and subcortical fc. This observation deviates from the reduced interhemispheric S1FL and thalamic fc after addition of a dexmedetomidine CRI to isoflurane reported in a study in rats. As the latter study combined doses used for imaging under monoanaesthesia with isoflurane or dexmedetomidine, the deeper level of anaesthesia may partially account for the decrease in fc. When isoflurane and (dex)medetomidine were compared in rats, correlation coefficients between ROIs as well as in seed maps were higher under isoflurane, but also less localised, and one study noted that segregation patterns in

ICA were more reproducible under medetomidine, leading to the general pattern of more specific, but weaker fc under medetomidine, which may have contributed as well to the reduced fc when dexmedetomidine was added to isoflurane.

Interestingly, studies in mice, all by the same group, found both in stimulation and resting state experiments similarities between urethane and medetomidine. Fewer and smaller activated clusters were detected under urethane and medetomidine than under isoflurane. ReHo, a measure of local connectivity, was under both agents relatively high. Cortical interhemispheric connectivity under urethane appeared to be higher than under medetomidine, but lower than under isoflurane. In rats, responses to phMRI were (at least in cortical regions) of higher magnitude than under isoflurane or medetomidine, but exclusively negative in one study. Regardless of its profile compared to other anaesthetics, urethane however appears to be an unfavourable candidate for fMRI studies due to its cycling behaviour. Additionally, it can only be used for terminal studies, and is carcinogenic and therefore an occupational safety risk for the handler.

Now that changes in physiological parameters can affect the BOLD signal as well as differences in anaesthetic protocols can, how can we know whether the differences between anaesthetic protocols, doses and timepoints of measurements were truly due to the anaesthetic factor and not biased by physiological parameter effects? The answer is that we can't. Indeed, in some references we noticed deviations in physiological parameters from normal range or differences in physiological parameters between two sets of measurements. However, our approach was not to dissect individual studies for suspected confounders, but to see whether, despite the heterogeneity of studies, effects of doses, timepoints of imaging or using a specific agent instead of another were detectable – with the reasoning that if there were effects detectable across a wide variety of experimental conditions and sources of bias, there likely is some true effect behind the reported findings, justifying careful further investigation.

4.3 Limitations

This systematic review was limited by the fact that only in a quarter of the included references data was extracted in duplicate. Despite good agreement in those references, some mistakes or misinterpretations made during data extraction may have gone undetected in the rest of the references, which could have been avoided by consequent double extraction. An even more important limitation is however that only one reviewer was available for study selection. Subjectively unclear references were discussed with a supervisor and all borderline cases documented, but this approach does not provide the same degree of objectivity as study selection by two independent reviewers. Additionally, in- and exclusion criteria had to be further specified during study selection because the initial version did not cover all the situations encountered. Especially for clarification of the exact inclusion and

exclusion criteria, discussion with a second reviewer would have supported objectivity and consistency of classifications.

On the side of included references, a major limitation of this review is that all included publications scored as having a high risk of bias. Accordingly, the strength of evidence is limited for any of the reported findings. The primary reason why studies were classified as having a high risk of bias was lack of blinding during the experiment and/or for data analysis. Lack of blinding during the experiment is common in basic research (van der Worp et al., 2005; Hooijmans et al., 2014; Macleod et al., 2015; Vogt et al., 2016). Often the same person is responsible for planning and performing the experiment and later analysing data. But even if it is not feasible for the responsible investigator to be blinded during the experiment due to lack of personnel or resources, fMRI data could be analysed in a randomised, blinded way (Hooijmans et al., 2014). Not a single publication mentioned randomised, blinded data analysis. Many stated instead that the pipeline of analysis was “fixed” or required minimal input from the operator and was thus free from bias. While (semi-) automated analysis may reduce bias to a certain degree, the pipeline of analysis is – based on personal experience – usually not completely pre-set but optimised once data is available. This fine-tuning leaves some room for bias. Apart from blinding, in a substantial percentage of publications concerns associated with study design were present, such as fixed order of conditions, inadequate crossover, differences in fluid administration between experimental groups (i.e. pooling of drugs) or insufficient detail of reporting of relevant aspects of study design. Reporting of measures against internal bias was – in line with published findings (Macleod et al., 2015) – generally low: aspects of sequence generation, allocation concealment and whether the animals underwent the experiment in a randomised order were rarely reported.

Risk of bias being high in all studies means that strength of evidence is overall weak, and it is possible that future research will complement or correct our current understanding of how physiological parameters and states of anaesthesia influence BOLD fMRI in rodents (Higgins et al., 2011; de Vries et al., 2015). A uniform level of risk of bias means further that consistency of findings for a certain comparison or factor is the only means to grade the strength of evidence between findings within this review. In the original systematic review protocol, we had planned to use the four criteria of risk of bias across included studies and consistency, directness and precision of findings to grade the strength of evidence (Owens et al., 2009; Berkman et al., 2015). This set of criteria was then reduced to risk of bias across studies and consistency of findings, because it became clear upon re-consideration that only studies providing direct evidence for effects of physiological parameters or states of anaesthesia would be included and that the precision of findings could hardly be assessed.

Besides concerns about general methodologic quality of the included studies, examples for potentially confounding factors addressed by this review were observed in some of the included studies. For example, Brynildsen et al. (2017) investigated evolution of responses to paw stimulation and fc over time after initiation of a dexmedetomidine CRI, but over the observation period respiratory rates increased in all animals and p_aCO_2 decreased on average from 62 to 50 mmHg in a bench-top

group. On the other hand, Nasrallah et al. (2015) compared responses to paw stimulation and fc under different inspiratory gas compositions, including several concentrations of CO_2, to a “normal” condition in which animals were ventilated to a p_aCO_2 of 27 mmHg, which is clearly below reference ranges (Brun-Pascaud et al., 1982).

In general, the included studies exhibited considerable heterogeneity regarding study design, experimental procedures (e.g. whether surgery was performed prior to imaging, whether anaesthesia was induced with another agent than maintained, respiratory management and (baseline) inspiratory gas compositions), magnetic field strength, and subjectively also in applied MRI sequences and data analysis, although those two aspects were not considered in data extraction. Although search filters were set for publication year 1990 or later, only few publications from the 1990s were included. As no limitations were defined for the outcome measures except that it had to be directly derived from the BOLD signal, a vast variety of outcome measures was encountered in the included studies. The original idea of differentiating between qualitative and quantitative outcomes was abandoned because it proved difficult to draw a clear line in individual cases: should a verbal description of extent of activation for example be considered as qualitative or quantitative measure?

The diversity of fMRI paradigms and primary outcome measures together with the diversity of anaesthetics combined for comparison, of doses of each anaesthetic and timeframes investigated, resulted in generally few reports per comparison and many unique reports. On one hand, this diversity narrowed the number of comparisons for which enough data was found to arrive at a conclusive summary. On the other hand, if despite all heterogeneity between studies an effect was consistently reported, it increases the likelihood that the reported observations are actually due to a real effect and not just the result of a biased study. It further means that an effect is relatively robust across conditions, which increases the external validity of that given effect.

4.4 Practical implications

Despite the discussed limitations, the available data indicate that BOLD fMRI measurements risk to be confounded by unstable physiology or unstable effective anaesthetic concentrations. Accordingly, results cannot be reliably compared between studies when different drugs, doses or time points of image acquisition are used and when p_aO_2, p_aCO_2 and MAP are not monitored and accounted for.

In the following section, practical implications of our findings are discussed together with general aspects of good anaesthetic practice.

4.4.1 Monitoring

Based on the findings of this review, fMRI studies should monitor oxygenation, ventilation and cardiovascular parameters. As part of good anaesthetic practice, reflexes and body temperature should also be monitored.

4.4.1.1 Ventilation and oxygenation

There are different options to monitor ventilation and oxygenation. Respiratory rate is commonly measured with MRI-compatible sensors. For anaesthetic safety, respiratory rate is an important parameter to monitor, because apnoea or severe respiratory depression can be fatal. In spontaneously breathing animals, respiratory rate additionally helps to estimate anaesthetic depth, with higher than expected rates typically indicating superficial levels of anaesthesia and lower than expected rates indicating (too) deep levels of anaesthesia, although other reasons for changes in both directions are possible and values as well as changes of respiratory rate need to be interpreted in context (a complete guide on how to interpret each physiological parameter in relation to all others is beyond the scope of this review). Although providing useful information, monitoring of respiratory rate alone is however insufficient if abnormal ventilation and oxygenation are to be excluded.

The most accurate way to assess ventilation and oxygenation is direct measurement of p_aCO_2 and p_aO_2 in arterial blood. However, this is invasive and the number of samples which can be taken before blood loss becomes substantial is limited. Substantial blood loss not only imposes physiological stress on the animal, but potentially affects the BOLD signal by inducing hypotension or by the resulting haemodilution when the withdrawn volume is replaced. An additional limitation of arterial blood sampling is that measurements are inherently intermittent. Depending on the duration of the experiment, the feasible number of samplings may not provide a sufficient temporal resolution. Approximately 0.1 ml of blood are required per analysis (Institute for Physiology, 2016) and maximally 10% of total blood volume should be sampled, which corresponds to 2.56 ml and 0.14 ml in a rat of 400 g and a mouse of 25 g (NC3Rs, 2019a,b). In mice, multiple samplings are therefore basically excluded.

Oxygenation can alternatively be monitored by pulse oximetry. Pulse oximetry is continuous, non-invasive, easy to use, and MRI-compatible devices are available. It provides real-time non-invasive monitoring of arterial haemoglobin O_2 saturation and has the additional advantage that heart rate is displayed along with SpO_2. Pulse oximetry is a powerful tool to ensure that animals are not hypoxaemic. Due to the sigmoid relation between p_aO_2 and SpO_2, it is however not able to differentiate between normal p_aO_2 observed at room air (around 100 mmHg) and increased p_aO_2 under hyperoxic conditions (up to 500 mmHg) (Duke-Novakovski et al., 2016). Grading of moderate levels of hypoxaemia (p_aO_2 60-90 mmHg) by pulse oximetry is also not very accurate, as those values correspond to SpO_2 of approximately 88 to 95% (Cartheuser, 1993).

As discussed earlier, optimal FiO_2 for BOLD fMRI in anaesthetised animals is likely in the range of 0.25 to 0.40. The inspired oxygen concentration provided should be continuously measured with a relevant gas analyser, that is calibrated at regular, by manufacturers predetermined, intervals.

Measurement of end-tidal CO_2 by capnometry or -graphy can be used as an estimate for p_aCO_2. It is important that capnographs designed for rodents are used (Beck et al., 2014). Due to small tidal volume of rodents, it is crucial that devices were calibrated against p_aCO_2 as significant discrepancies can be found, documented for example in Nasrallah et al. (2015). To obtain accurate measures, animals need to be intubated. A theoretical alternative to capnometry is the use of transcutaneous pCO_2 measurement. Such a transcutaneous sensor to detect blood pCO_2 was tested in one of the included studies (Ramos-Cabrer et al., 2005). Due to considerable inter-individual differences in correlation with p_aCO_2, dependence of measurements on skin perfusion, and the risk for skin lesions due to the high temperature of the sensor reported in that study, we currently discourage use of transcutaneous pCO_2 sensors.

Another theoretically possible approach to control p_aCO_2 is to ventilate all animals with settings determined in pilot bench-top experiments. While this approach may on average keep p_aCO_2 and p_aO_2 within reasonable limits, it requires first that the ideal parameters are determined under identical conditions as in the experiment and second has the major limitation that eventual outliers are not detected when no additional monitoring is used. Notably when low animal numbers are used, individual outliers may have a relevant impact on the results if undetected (which is a general problem of measuring physiological parameters in a bench-top group only). Additionally, if the pre-set ventilatory parameters do not exactly match individual requirements, hyper- or hypoventilation can develop over time. While ventilating allows to control p_aCO_2, adequate monitoring (i.e. blood gas analysis or capnography) must be used to confirm that CO_2 levels are maintained within a target range. We discourage the use of artificial ventilation without monitoring of end-tidal CO_2.

A practical consideration with mechanical ventilation is that neuromuscular blocking agents are often required to prevent animals from “fighting” the ventilator (Ramos-Cabrer et al. (2005). In a clinical setting the degree of neuromuscular blockade is monitored, for example by train of four electrical stimulation, to avoid residual paralysis when animals are recovered (Duke-Novakovski et al., 2016). This is however difficult in rodents due to their small size and unlikely to be routinely used in the laboratory setting, which means that animals are at increased risk for respiratory complications during recovery, due to residual neuromuscular block.

4.4.1.2 Cardiovascular monitoring

As shown by the effects of blood pressure changes on the BOLD signal, blood pressure needs to be measured in anaesthetised animals during fMRI. To date, blood pressure is typically measured invasively by catheterization of an artery which is in particular in mice technically demanding, time consuming and probably not

practicable if multiple scanning sessions are planned in one individual. MRI-compatible non-invasive methods of blood pressure monitoring are available for rodents (e.g. systems from IITC Inc. Life Science), were however not used in any of the included studies. If measurements acquired with non-invasive devices proof to be accurate, the quality of anaesthetic monitoring could be improved without increasing invasiveness of an experiment.

Although heart rate was not shown to directly correlate with BOLD signal, it should be monitored during fMRI, as changes in heart rate can indicate responses to stimulation, changes in anaesthetic depth or also changes in p_aCO_2 and p_aO_2. Heart rate, similar to respiratory rate, is a sensitive, but not specific parameter and therefore needs to be interpreted in the context of other physiological parameters, experimental stimulation and the anaesthetic protocol used. A convenient method to measure heart rate is by pulse oximetry. Alternatively, MRI-compatible ECG electrodes can be used (Choquet et al., 2011).

Some pulse oximeters display a pulse curve. Schroeter et al. (2014) used changes in pulse distension, i.e. the waveform amplitude, as an indicator for changes of blood pressure. However, this specific measure was not validated and should be interpreted with caution. Generally, variation in the waveform amplitude measured by the pulse oximeter results from an interplay between vascular resistance and stroke volume (Cannesson et al., 2005). Assuming constant vascular resistance over a short period of time (in the range of a few seconds), relative changes in the waveform amplitude indicate a change in stroke volume. This may be sufficient to detect cardiovascular arousal as intended in that study. Inferences on – absolute or relative - arterial blood pressure changes are however not warranted (Dorlas and Nijboer, 1985) and pulse oximetry can therefore not replace blood pressure monitoring.

4.4.1.3 Additional considerations

In terms of good anaesthetic practice, it is furthermore advisable to monitor depth of anaesthesia and temperature.

Reflexes give a rough clinical estimate of the depth of anaesthesia. Before an animal is fixed on the animal holder, righting and limb- or tail-withdrawal reflex should be tested, especially if agents were administered ip, as this route is associated with high failure rates of around 20% (Miner et al., 1969; Zatroch et al., 2017) so that the same levels of anaesthesia will not be reached in all animals. The desired depth of anaesthesia may vary depending on the protocol used, but at least righting reflex should be lost under exposure to scanner noise. Clinical experience from cats and dogs suggests that levels close to surgical anaesthesia are required due to the intense stimulus of scanner noise. Reflexes should be checked again at the end of the scan to recognise changes in anaesthetic depth which may have occurred during image acquisition; especially if injection anaesthesia is used.

Vanhoutte et al. (2006) showed that BOLD signal intensity decreases when animals are experimentally warmed over 38° C. This effect is relevant when stroke models are imaged, as spontaneous hyperthermia can occur in those animals (Zaremba,

2004). Body temperatures reported by included studies however typically ranged from 35.5 to 37.5° C, and no data was available on temperature-dependent effect on the BOLD signal in this range. As cerebral metabolism is reduced (Busto et al., 1987) and the O_2-binding curve of haemoglobin shifted to the left (Armstrong et al., 2005) at lower body temperatures, higher blood oxygenation and accordingly BOLD signal intensity would be expected at lower body temperatures, but the true effects remain to be investigated. Regardless of effects on the BOLD signal, hypothermia has detrimental physiological effects as bradycardia and hypotension, prolongs recovery and reduces anaesthetic requirements (Armstrong et al., 2005). Rats and mice are prone to hypothermia under anaesthesia due to their small body size. To prevent complications, the decrease in body temperature should be minimised by warming the animal, for example with feed-back controlled MRI-compatible warming mats, and body temperature monitored (Flecknell, 2015).

4.4.1.4 Summary

Taken together, appropriate monitoring for a BOLD fMRI experiment is continuous, invasive and not simple. To control p_aCO_2, probably the most important factor, either blood samples have to be drawn or the animals have to be intubated to allow accurate capnography. Mechanical ventilation is generally recommended to keep p_aCO_2 constant. Mechanical ventilation of rodents via a nose-mask is described (Rindfield and McBrian, 2012). However, in 12% of cases the lung was not successfully ventilated with this option. If refinement of this method proves feasible in further studies and allows accurate monitoring of end-tidal CO_2, mechanical ventilation via nose-mask could provide a less invasive and technically less demanding alternative to intubation.

For blood pressure monitoring to be more accessible and generally applied, validation and/or development of accurate non-invasive blood pressure measurement devices for rodent MRI should be a priority. Reliable non-invasive blood pressure monitoring would allow BOLD fMRI studies to be non-invasive but at the same time sensitive enough to recognise any potentially relevant blood pressure changes during the experiment.

4.4.2 Anaesthetic protocols

When it comes to choosing an anaesthetic protocol for BOLD fMRI, not only the agents to be used, but also the doses, the timing and practical aspects must be considered.

Generally, steady state anaesthesia is easier to reach and notably to maintain for prolonged periods with inhalants. The included studies investigated most commonly isoflurane and occasionally halothane. None of them investigated sevoflurane. As sevoflurane causes less increase of CBF than isoflurane and better maintains cerebral autoregulation (Hänel et al., 1996; Gupta et al., 1997; Lenz et al., 1998), it is the inhalant of choice for clinical procedures in neurological patients and would be

worth further investigation for rodent fMRI. For specific experimental paradigms, the benefits of total or partial injectable anaesthesia may however outweigh the advantage of steady state under inhalant anaesthesia. For example, injectable anaesthesia protocols including α_2-agonists may be preferable for sensory stimulation studies in mice. The fact that already sub-anaesthetic doses of ketamine produce distinct activations illustrates that agent-specific effects have to be expected and, when known, considered when designing the anaesthetic protocol for an fMRI experiment. In the special case of phMRI, it is crucial to avoid anaesthetic agents which might interact with the tested substances. Potential interactions have to be identified for each tested substance individually.

Once the anaesthetic(s) for the experiment have been chosen, it must be decided whether the same agent(s) can be used for induction of anaesthesia and preparation. Generally, switching from one anaesthetic to another bears the risk of carry-over effects, especially when injectables are used, but also if appropriate wash-out periods are not awaited when switching from inhalant to injectable anaesthesia. Situations in which anaesthesia induction with a different agent than later used for the experiment is justified are when preparation of the animals includes surgery and the anaesthesia planned for imaging would be too superficial for surgery (e.g. a (dex)medetomidine CRI) or when the selected agent is not a suitable induction agent, (e.g. α-chloralose).

Doses should be sufficient to provide a stable level of anaesthesia, but not higher to avoid excessive neural depression. If doses are too low, the animals are cardiovascularly less stable and more likely to be aroused by experimental stimulation or noise from the MRI sequence (Constantinides et al., 2011; Schroeter et al., 2014). Optimal doses should be determined under conditions which closely mimic the experimental conditions, including scanner noise. Uptake of injectable agent varies between routes of administration (sc, ip, iv) which may affect both the required doses and the half-life time. Importantly, not all routes of administration are suitable for all agents. While medetomidine can be administered sc, ip or iv, propofol for example has to be administered strictly iv (Becker et al., 2016).

When animals of variable body weight are studied, individual dosing of injectable agents should be aimed for. For example, if a fixed total dose of anaesthetic is administered to rats of 300 g to 400 g bodyweight, a 25% difference in relative dose results.

In order to keep doses as low as possible and therefore to reduce unwanted effects, anaesthetic agents can be combined. The concept of balanced anaesthesia, i.e. the combination of agents to use synergistic effects while minimising agent specific undesired effects, is well established in clinical anaesthesia (Tonner, 2005). A balanced anaesthesia protocol combining low doses of isoflurane and medetomidine was shown to preserve cortical as well as subcortical connectivities in mice, whereas monoanaesthesia with a higher dose of either agent favoured either cortical or subcortical connectivity (Grandjean et al., 2014). The same authors noted that differences in fc patterns between four different anaesthetic agents were less pronounced when a lower dose of each agent was used, which can be explained by weaker contribution of the respective agents to haemodynamic as well as neural

aspects of the BOLD signal. Balanced anaesthesia for BOLD fMRI may thus allow to obtain data which is more representative of normal brain function and therefore easier to translate to awake humans. A potential disadvantage of balanced anaesthesia is that the pharmacokinetics and -dynamics get more complex the more agents are involved. This should however rather en- than discourage thorough investigation of potential protocols.

Whenever protocols are established or existing protocols optimised, a focus should be the time span during which anaesthesia reaches or at least approaches steady state. Only if anaesthesia is close to steady state throughout image acquisition, reliable data can be obtained. Additionally, if anaesthesia is induced with an inhalant agent and later switched to injectable agents, the duration of inhalant anaesthesia has to be standardised and measured.

As a general rule, the time taken for each step of an experiment should be documented for each animal and standardised as much as possible. Articles should report timepoints of image acquisition relative to induction, eventual boli and start of CRIs.

Finally, a few more factors need to be considered:

If preparation of the animal includes any surgical steps, adequate analgesia should be provided to prevent measuring activations from nociception. For catheterisation of vessels, topical lidocaine appears a reasonable choice. For more invasive procedures such as craniotomies, additional analgesia will typically be required and the selection of agents depend on analgesia provided by the underlying anaesthetic protocol.

Atropine or glycopyrrolate are best avoided in premedication when boli of α_2-agonist are administered, because they suppress (protective) reflex-bradycardia in the first few minutes of vasoconstriction after a medetomidine bolus, which can be fatal (Savola, 1989). Furthermore, fading concentrations of those parasympatholytics counteract cardiovascular stability during imaging.

It is important to ensure that animals from all experimental groups receive the same total amount of fluids, including the calculation of drugs and dilutions of such as well as catheter flush solutions. As α_2-agonists cause pronounced diuresis (Roman et al., 1979; Gellai and Edwards, 1988), expected fluid losses should be replaced.

Last but not least, important strain and sex differences in responses to anaesthetics are reported in rats (Waterman and Livingston, 1978; Fink et al., 1982; Russell and Graybeal, 1995; Avsaroglu, 2008) as well as in mice (Hohlbaum et al., 2017; Schroeter et al., 2017). Researchers should therefore search for information on strain-specific sensitivity to anaesthetics when designing a protocol and eventually adjust doses.

We suggest establishing a selection of standard protocols for the most commonly used agents to increase the comparability and reproducibility of studies. There will always be an effect of anaesthesia on the measurements, but awake imaging is not confounder free either: animals were stressed during actual imaging session even

after extensive training in snuggle bags (Chang et al., 2016). If performed properly, training animals to voluntarily enter snuggle bags and tolerate such a set-up for restraint may still be an alternative to anaesthetised imaging in rats, but we doubt that this time consuming technique is broadly applicable because it requires a level of skills and motivation which cannot be taken for granted in all laboratories. An additional limitation to awake imaging is that to our knowledge an equivalent technique has not been described in mice so far, which are generally considered to be more difficult to train. From an animal welfare point of view, imaging under anaesthesia is therefore preferable in most circumstances. Well-designed anaesthetic protocols, based on pilot studies under realistic conditions (e.g. with scanner noise), and careful monitoring are crucial to obtain interpretable, reproducible and comparable fMRI results.

4.5 Systematic reviews in basic science

In basic (life) science, systematic reviews are not common yet. A major challenge in the present review was the heterogeneity among the included studies – not only concerning details of the experimental set-up, but also in the primary research question addressed by those studies. In a relevant proportion of references, the primary aim of the study was not to investigate effects of physiological parameters or anaesthetic protocols. Those effects were either included as a secondary aim (to demonstrate that an effect is reproducible in different conditions) or used to investigate specific aspects of the BOLD signal.

This heterogeneity among studies partially results from the broadness of our research question, asking for the feasibility of a method in general rather than for the safety or efficacy of a specific intervention as in prototypic pre-clinical systematic reviews. Despite the broadness of the question, we considered this a systematic and not a scoping review, because we aimed to provide implications for practice (Munn et al., 2018). Additionally, we speculate that the diversity of questions addressed by original studies within a certain field and the resulting heterogeneity are typical for basic science and a challenge to broader application of systematic reviews in basic science.

Nevertheless, basic science could profit from attempts to synthesise the state of evidence systematically rather than in a narrative way as is the current standard: the systematic literature search avoids bias towards much-cited articles and makes sure that all available evidence is considered. A systematic review is therefore less prone to being dominated by personal hypotheses than a narrative review. If a meta-analysis can be performed, systematic reviews additionally give a more realistic estimate of true effect directions and magnitudes. Systematic reviews allow “to get out the maximum” from research that has already been done on a subject while also critically assessing the quality of available evidence and are a powerful tool to plan further research (de Vries et al., 2015). This means that resources and animals can

be allocated more efficiently and contributes to reducing the number of animals in experiments.

Systematic reviews are greatly facilitated when original reports precisely and transparently report their methods and results. The ARRIVE guidelines to improve reporting in bioscience were published in 2010 (Kilkenny et al., 2010). Despite endorsement by numerous journals and funding agencies, reporting has not markedly improved thereafter, and the guideline is currently under revision to make it more user-friendly (Percie du Sert et al., 2018). Both the lack of clear statements on the significance of results in some studies and unclear descriptions of study design and/or methods made it more difficult for us to compare findings between studies. To enable systematic reviews in basic science, funding agencies, committees licensing animal experimentation and journals should stress that studies are designed and reported in a way that they are later eligible for systematic reviews. In the field of fMRI, this includes reporting of the timeline of the experiment, notably the timing of image acquisition relative to induction and eventual switch to other agents and/or inhalant concentrations or infusion rates, in addition to the anaesthesia-related item listed in the ARRIVE guidelines. Finally, registration of all animal studies would have helped to keep track of which data was re-analysed or newly acquired in the cluster of references described in chapter 3.2, and would in general increase transparency.

Clinical systematic reviews and meta-analyses typically exclude original reports with a high risk of bias or stratify results in the meta-analysis by risk of bias (Higgins et al., 2011). As it was anticipated that many studies would be at high risk of bias, we decided to not exclude those, and indeed, all included references scored as being at high risk of bias. The SYRCLE risk of bias tool which we used for this review (Hooijmans et al., 2014) was developed on the base of the Cochrane risk of bias tool to assess randomised clinical trials (Higgins et al., 2011). While it is true that for most experimental animal studies there is no good reason why they should not be randomised and – at least for outcome assessment – blinded, to date most studies are not. This results in studies being automatically classified as at high risk of bias by the SYRCLE risk of bias tool. Subtle differences in methodological quality between studies with overall suboptimal design are therefore not caught. Although such a differentiation would not have changed the overall conclusion that the results of our review are likely to be complemented or corrected by further research, appreciating subjectively noted differences in the risk of bias tool could support objectivity of a systematic review. Until reporting and general scientific quality of animal studies improve, systematic reviews of animal studies will have to discuss how to deal with the endemic high risk of bias.

4.6 Implications of our findings for BOLD fMRI validity in rodents

As all animal experimentations, rodent fMRI studies must undergo harm-benefit analysis. Studies can only be justified when the anticipated benefits outweigh the

harms. However, for studies to generate any benefit, the results have to be scientifically valid (Würbel, 2017). Würbel (2017) proposed to assess the scientific validity of studies by the three aspects of construct, internal and external validity.

Construct validity was previously defined by these authors as “The degree to which inferences are warranted from the sampling properties of an experiment (e.g., units, settings, treatments and outcomes) to the entities these samples are intended to represent” (Würbel et al., 2014). The construct validity of a rodent model using BOLD fMRI for outcome assessment depends on the purpose and the specifications of the model used. A detailed assessment of the variety of models in use is beyond the scope of this review. However**,** a question to be raised when rodents are used to model complex behavioural and/or emotional functions of the human brain is whether imaging anaesthetised animals can provide relevant information. If the answer is no, the next question is whether relevant information could be acquired by imaging animals in the awake state. As discussed above, awake imaging should only be performed in animals which were trained to voluntarily enter and tolerate the restraint set-up to minimise confounding effects of stress. Unless animals are carefully trained, awake imaging should generally be considered to be at a high risk for confounding effects of stress and/or effect of repeated anaesthesia and/or anaesthesia used for positioning of the animal. If neither anaesthetised nor awake imaging is anticipated to allow capturing the state of interest, performing the experiment is not warranted (insufficient construct validity).

Internal validity was previously defined as “The extent to which the design, conduct, and analysis of the experiment eliminate the possibility of bias so that the inference of a causal relationship between an experimental treatment and variation in an outcome measure is warranted” (Würbel et al., 2014). Regarding the use of anaesthesia, it is difficult to draw a clear line between construct and internal validity: For specific models, whether relevant information can be acquired from anaesthetised animals may well depend on the anaesthetic(s) and even doses used. Assuming that construct validity is given for a certain rodent fMRI study using a certain anaesthetic agent, the timing of image acquisition as well as monitoring and control of physiological parameters are part of the conduct of the experiment and therefore relevant for the internal validity of a study. Neglecting potential effects of physiological parameter alterations and the chosen anaesthetic protocol (including timing) on the BOLD signal introduces bias and thus reduces the internal validity.

External validity is generally defined as the extent to which findings can be generalised (Würbel et al., 2014). While it is true that over-standardisation reduces external validity and consequently reproducibility of research findings, we strongly believe that heterogeneity should be included in studies in a controlled, systematic way and not by failure to control for potential confounders.

Establishing standards of monitoring, as well as evidence-based optimal dose ranges and imaging timepoints for a selection of anaesthetic protocols is therefore a priority for improving the scientific validity of rodent fMRI studies.

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6 Appendix

6.1 Abbreviations and glossary

MRI – magnetic resonance imaging

fMRI – functional magnetic resonance imaging; several modalities exist

BOLD – blood oxygen level dependent

BOLD fMRI – an fMRI modality weighted for blood oxygen level dependent contrast

T2* – “observed” or effective transverse relaxation time, resulting from the combination of “true” transverse relaxation time T2 and magnetic field homogeneities

R2* – “observed” or effective transverse relaxation rate, resulting from the combination of “true” transverse relaxation rate R2 and magnetic field homogeneities

ΔR2* – difference in R2* between two timepoints or states

rs – resting state: in animals defined as absence of external stimulation, in humans additionally defined as a state of “unrestrained cognition”

rsfMRI – resting state fMRI: acquiring fMRI of a subject in the resting state. Subsequent analysis relies on slow frequency fluctuations of the BOLD signal (<0.1 Hz). (Smitha et al., 2017)

phMRI – pharmacological MRI: the use of fMRI to investigate central effects of test substances, in this review specifically investigation of central drug effects by BOLD fMRI

ROI – region of interest

fc – functional connectivity: “interaction between two different brain regions (…) inferred on the basis of correlations among the parameters of neuronal activity” (Smitha et al., 2017)

ICA – independent component analysis: blind source separation algorithm that decomposes signal fluctuations of all voxels of the brain in spatially and temporally independent components (Smitha et al., 2017)

ReHo – regional homogeneity: similarity of each voxel’s signal time course with the signal time courses of its closest neighbours (Smitha et al., 2017)

CAP – co-activation pattern: fc is analysed by clustering activation patterns from individual frames (Liang et al., 2015a)

$CMRO_2$ – cerebral metabolic rate of oxygen

CBF – cerebral blood flow

CBV – cerebral blood volume

S1 – primary somatosensory cortex

S1FL – frontlimb area of the primary somatosensory cortex

S1HL – hindlimb area of the primary somatosensory cortex

S1BF – barrel field of the primary somatosensory cortex, area receiving input from whiskers

S2 – secondary somatosensory cortex

M1 – primary motor cortex

M2 – secondary motor cortex

CPu – caudate putamen

VPM – ventral posteromedial nucleus of the thalamus

VPL – ventral posterolateral nucleus of the thalamus

sc – subcutaneous

ip – intraperitoneal

iv – intravenous

CRI – constant rate infusion

O_2 – oxygen

N_2 – nitrogen

N_2O – nitrous oxide

CO_2 – carbon dioxide

FiO_2 – fraction of inspired oxygen

pCO_2 –partial pressure of carbon dioxide

p_aO_2 – arterial partial pressure of oxygen

p_aCO_2 – arterial partial pressure of carbon dioxide

MAP – mean arterial blood pressure

Hypoxia – low partial pressure of O_2 and O_2 content in tissues (MacIntyre, 2014)

Hypoxemia – $p_aO_2 < 80$ mmHg (Grimm, 2015)

Hyperoxemia – $p_aO_2 > 110$ mmHg (Grimm, 2015)

Hypercapnia – $p_aCO_2 > 45$ mmHg (Grimm, 2015)

Hypocapnia – $p_aCO_2 < 35$ mmHg (Grimm, 2015)

SD – standard deviation

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6.2 Systematic review protocol: The impact of anaesthesia protocols on BOLD fMRI validity in laboratory rodents

As described in the materials and methods section, the study design was adapted during the initial phase of the review. For completeness, the original study design, as planned on the day the systematic search was conducted, is shown in the following paragraphs.

6.2.1 Stage of the review at time of registration

An initial systematic search was performed, and records were selected and partially analyzed by a single author, when it was decided to update the search with refined methodology.

6.2.2 Objectives

Blood oxygen level dependent (BOLD) fMRI is the most commonly used modality for functional neuroimaging in humans (several thousand studies published per year) and widely used in preclinical and basic research rodent models (Martin, 2014; Jonckers et al., 2015; Pan et al., 2015). Changes in blood oxygenation levels are interpreted as a surrogate for neuronal activation, based on the mechanism of neurovascular coupling (i.e. activation of neurons increases local blood flow) (Logothetis and Wandell, 2004). As even small movements of the head during image acquisition distort the images, animals are typically anaesthetized for image acquisition. In terms of animal welfare, scanning under anaesthesia is preferable to awake scanning under rigorous fixation (Low et al., 2016). However, anaesthesia interferes with interpretation of the BOLD signal on several levels: first, alterations in physiological parameters (e.g. hypotension, hypercapnia) can alter local hemodynamics in absence of changes in neuronal activity. Second, anaesthetics per definition alter neuronal activation and information processing, so that measurements may reflect activation under a distinct anaesthetic rather than universal patterns. Third, anaesthetics may modulate signal cascades responsible for neurovascular coupling. In practice, it is difficult to clearly separate the relative contribution of each mechanism. Therefore, one part of the review will focus on studies which directly compare different anaesthetic protocols with each other or with awake scanning. We want to analyze how different states of anaesthesia affect the BOLD outcome measures specified in the respective studies in adult rats and mice. States of anaesthesia are defined as distinct if they differ by the drug or dose administered or by the time that has elapsed since induction. The other part of the review will focus on the specific effects of alterations in physiological parameters on different BOLD outcome measures in adult rats and mice. Physiological parameters that can typically be altered under anaesthesia are p_aCO_2, p_aO_2, SpO_2, respiratory rate, respiratory pattern, heart rate, arterial blood pressure and body temperature.

Due to the vast diversity of post-processing options in BOLD fMRI, any outcome measure specified by the individual studies is eligible for analysis, as long as the study uses BOLD contrast on the brain parenchyma (whole brain or specific regions of interest) in order to draw a conclusion about functional aspects of the brain.

Analysis will include studies conducted in healthy animals as well as studies in disease models, as emphasis is placed on providing researchers with relevant information for deliberately choosing an appropriate anaesthetic protocol for BOLD fMRI experiments in laboratory rodents.

In short, our research question reads as follows: What is the effect of a) different states of anaesthesia or b) changes in physiological parameters that can be observed under anaesthesia on the BOLD fMRI outcome measure defined by the individual study in adult rats and mice?

Our aim is to demonstrate the extent of anaesthetic protocol-related differences in fMRI outcomes, to formulate evidence-based minimal standards for monitoring during BOLD fMRI experiments, and ultimately to elaborate recommendations on how to choose an appropriate anaesthetic protocol. To our knowledge, this is the first systematic review about the impact of anaesthetic protocols on BOLD fMRI validity in laboratory rodents.

6.2.3 Methods

6.2.3.1 Search strategy and study identification

A systematic search will be conducted in EMBASE, MEDLINE, Scopus and Web of Science. Search terms are listed in the table below. Search terms within one block are linked with “OR”, while the blocks are linked with “AND”. Terms will be searched in titles and abstracts. Correspondent Emtree vocabulary and MeSH, if available, will additionally be used for the search in EMBASE and Medline, respectively. Language will be restricted to English, German and French. As the first publication describing BOLD contrast in MRI appeared in 1990, a filter for publication year 1990 or later will be used.

Rodents
rat OR rats OR mouse OR mice OR rodent OR rodents
MRI
((MRI OR MRT OR NMR OR “magnetic resonance imaging”) *proximity operator*5 functional) OR fMRI OR BOLD OR “Blood oxygen level dependent”
Anaesthesia OR physiology
anesthe* OR anaesthe* OR hypercapnia OR hyperoxia OR hypoxia OR apnea OR “blood pressure” OR hypotension OR hypertension OR autoregulation OR thermoregulation OR “physiological noise” OR “functional connectivity” OR somatosensory OR stimulation OR isoflurane OR sevoflurane OR halothane OR medetomidine OR dexmedetomidine OR alpha-chloralose OR chloralose OR a-chloralose OR urethane OR propofol OR ketamine OR xylazine

Additionally, any publication fulfilling inclusion criteria which is cited in reviews or original articles, but was not found by the systematic search, will be included.

If a selected study is recorded in the format of a proceeding/abstract/poster, google scholar will be used to search for a corresponding full article (for more details, see study selection).

6.2.3.2 Study selection

All records will be imported to endnote and duplicates removed with the “find duplicates” function. In a pre-screening phase, titles and abstracts will be screened. Records deemed eligible for analysis in the preliminary search automatically pass this stage. Records who have made it through pre-screening will undergo full text screening for eligibility. Study selection will be performed by a single reviewer. As there is not enough evidence to claim that study selection is performed by two reviewers (Shamseer et al., 2015), having a single reviewer performing this step can be justified against the common recommendations of having two reviewers independently screening the search results.

Inclusion and exclusion criteria are defined as follows:

	Inclusion criteria	Exclusion criteria
Type of study	Describing original research, reported in an article, abstract, conference proceeding or poster	Review; data originating from different experiment which is not described in material and methods.
Type of animals	Adult rats and mice of any strain. Adult defined as sexually mature, i.e. >=12 weeks or 200 g for rats; >= 8 weeks or at least 18 g for mice. For both species the lower limit of reproductive activity of 12 months is used as the upper age limit (Wolfensohn and Lloyd, 2013). If articles report to use adult animals, but do not specifiy the age or weight, studies are included. If they report to used adult animals but age and/or weight of some animals (e.g. if a range is given) are within 10% of deviation from the limits defined here, the	Other species; neonate/juvenile/geriatric animals, if a) defined as such by the study or b) not explicitely described as adults and not fulfilling the age and weight limits defined under inclusion criteria

	study is still eligible. Disease models are eligible.	
Type of intervention	BOLD fMRI a) Comparison of anaesthetic protocols or anaesthetized vs. awake for same imaging protocol b) alteration of physiological parameters: either deliberately caused by an intervention or closely monitored over time with the explicit intention (mention in abstract) of analyzing the correlation with fMRI signals. Acceptable paradigms: resting state; peripheral sensory stimulation (e.g. electrical, mechanical, chemical, visual, auditory or olfactory stimulation) including noxious stimuli; direct brain stimulation (e.g. electrical, optogenetic; chemical if specifc neurotransmitter systems are blocked/enhanced in order to elucidate the effects and mechanisms of action of anaesthetics)	fMRI applied to other regions of the body than brain (e.g. spine, heart, joints); modalities of fMRI other than BOLD (e.g. arterial spin labeling, CBV measurements); BOLD fMRI studies neither comparing states of anaesthesia nor investigating alterations of physiological parameters; pharmacologic stimulation for other purposes than elucidating the effects of mechanisms of action of anaesthetics by blocking/enhancing specific neurotransmitter systems.
Outcome measures	As defined by the respective study as long as BOLD contrast is used on the brain parenchyma to make a statement about functional aspects of the brain.	Correlations of BOLD signal with other functional neuroimaging methods, measurements of neural activity or cerebral hemodynamics
Language restrictions	English, German, French	All other languages

Publication date restrictions	1990 and later	Earlier than 1990
Other	-	Duplicate not recognized by endnote; strong suspicion of multiple reporting

In the title and abstract screening phase, priority exclusion criteria are non-rodent species, applications of fMRI to other regions of the body, non-BOLD fMRI modalities and reviews. In the full text screening phase, the focus is on excluding records which neither compare anaesthetic protocols nor investigate the effect of alterations in physiological parameters or which use pharmacologic stimulation for other purposes than elucidating the effects of mechanisms of action of anaesthetics by blocking/enhancing specific neurotransmitter systems, and data originating from a different experiment which is not described in the material and methods section of the present publication. If a study describes multiple interventions and outcome measures, only data from those experiments meeting the inclusion criteria will be analyzed.

Duplicates not recognized by endnote (e.g. due to different recording of author names in different databases) will be manually resolved. If a proceeding/abstract/poster and a full article by the same author reporting the same experimental protocol with the same primary outcome are found, so that the full article most probably describes the same study as the shorter form of publication, only the full article is kept. In order to get the best information available about individual studies, we will try to find a corresponding full article on google scholar for each short form record, using a combination of author names, keywords and restriction of the publication date to the first 3 years following the shorter publication. If a full article is found, the shorter publication is replaced, otherwise it is kept.

DistillerSR will be used to assist the screening and data extraction process.

6.2.3.3 Study characteristics to be extracted (for assessment of external validity, reporting quality)

For each study, animal characteristics (species, sex, strain, age, weight, number per experimental group), the exact anaesthetic protocol (drug, dose, route of administration, time point of administration, gas mixture, flow, concentration of inhalant anaesthetic), surgical preparations for the experiment, presence or absence of monitoring of specific parameters and details on the methods and frequency of monitoring (temperature, heart rate, oxygen saturation, respiratory rate/ventilator setting, end-tidal gas concentrations, blood gas parameters and timing, arterial blood pressure, reflexes) are extracted. For records investigating the effect of physiological parameters on BOLD fMRI outcome, details of the interventions to alter the physiological parameter(s) are additionally extracted. For all studies, magnetic field strength, duration of image acquisition and total experiment, type and timing of stimulations during BOLD fMRI, primary and secondary fMRI outcome as well as a brief description of additional methods used in that study are recorded. A separate

column allows to comment on specific issues of the study that are not covered by the risk of bias tool (e.g. inadequate methods, failure to discuss potential confounders).

6.2.3.4 Assessment of risk of bias (internal validity)

One reviewer will assess the risk of bias in each study.

An adapted version of SYRCLE's risk of bias tool (ROB tool) will be used. As only one reviewer assesses the risk of bias, a first version of the ROB tool will be sent to experts from several fields (welfare of laboratory animals, anaesthesia, fMRI), together with the original ROB tool and 2-3 representative publications. Their feedback will be integrated and, if major revisions are made, a revised version sent out for a second round of feedback, the primary question being "Do you think that this version of the adapted risk of bias tool is more adequate than the previous?".

6.2.3.5 Collection of outcome data

A peculiarity of our study is that the outcomes are heterogeneous; any outcome measure defined by an individual study is eligible as long as it results from analysis of BOLD fMRI and its purpose is to measure a functional characteristic of the brain.

For each study it will be extracted whether a) a qualitative and b) a quantitative difference was observed between different states of anaesthesia or different values of physiological parameters (values of physiological parameters are considered different if they are defined as different by the respective study). If a difference was observed, it will be specified. For physiological parameters, the absolute values of baseline, change and altered state will be extracted.

A second reviewer will control whether data was extracted correctly because mistakes are common at this step of the workflow (Shamseer et al., 2015).

6.2.3.6 Data analysis/synthesis

Data will be analyzed strictly separated for each species, but following the same structure of analysis.

6.2.3.6.1 State of anaesthesia

Per imaging paradigm, it will be analyzed whether studies comparing the same anaesthetics or dosages of the same anaesthetic first consistently report the presence or absence of qualitative differences between different states of anaesthesia, and second, if qualitative differences are observed, whether they are consistent, complementary or conflicting. An analog analysis will be performed for quantitative differences. For each comparison, a synthesis will be formulated, and the quality of evidence indicated.

Comparisons for which only single reports exist will be described as such.

Depending on the number of records about comparisons of states of anaesthesia in disease models, those records will be analyzed in a separate subchapter or along

with studies performed in healthy animals, but it will be analyzed whether the presence and/or nature of observed differences are correlated with the health status of animals under investigation.

6.2.3.6.2 Physiological parameters

Per physiological parameter, it will be analyzed whether in- and decreases, respectively, are consistently reported to affect BOLD outcome measures, which will be grouped in the same imaging paradigm categories as used in the state of anaesthesia section. For changes in PaCO2 and PaO2, the constitution of inspired gases will also be considered. If qualitative or quantitative differences are reported, it will be analyzed whether they are consistent, complementary or conflicting. It will be further analyzed whether the anaesthetic used or the source of change of the physiological parameter correlates with the presence and/or nature of observed effects. If enough data is available, it will additionally be analyzed whether the changes in BOLD signal are correlated with the magnitude of change of the physiological parameter's value.

For each parameter, it will be concluded whether it potentially affects BOLD measurements if unstable. The strength of evidence will be indicated.

Meta-analysis will be considered, but a definitive decision whether it will be suitable cannot be made yet.

6.2.3.7 Grading of evidence

For each subcategory, the strength of evidence will be graded according to the evidence-based practice center approach (EPC) (Owens et al., 2009). The following categories will be assessed across records: risk of bias (low/moderate/high), consistency (yes/no for direction of effect and effect size), directness (direct/indirect) and precision (precise/imprecise). Together, those assessments result in the classification of the body of evidence for that specific subcategory as high, moderate, low or insufficient.

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6.3 Inclusion and exclusion criteria

	Inclusion criteria	**Exclusion criteria**
Type of study	• original research • short form (conference abstract, poster or paper) or full article • re-analysis of previously acquired data	• review • opinion piece • book chapters • lecture/talk • complete congress proceedings/abstract collections • study protocol • short form with corresponding full article • multiple reporting • unrecognized duplicate
Type of animals (all limits refer to the timepoint of image acquisition)	• rats >200 g or 8 weeks and up to 12 months • mMice >18 g or 8 weeks and up to 12 months • if age/weight not described: assumed that animals were adult unless stated otherwise • both sexes • any strain • any health status	• species other than rat or mouse • rats <200 g or 8 weeks or >12 months • mice <18 g or 8 weeks or >12 months • if age/weight not described: animals described as pups/ neonatal/ juvenile/ adolescent/ geriatric/ aged/ old…
Type of interventions	BOLD fMRI of the brain with • comparison of different drugs, doses or timepoints of imaging relative to induction, or anaesthetized versus awake for same imaging protocol • alteration of physiological parameters: either deliberately caused by an intervention or closely monitored over time with the explicit intention (mention in abstract) of analyzing the correlation with fMRI signals. Accepted experimental paradigms • resting state • central stimulation paradigms	• no MRI • other (f)MRI modalities • other body regions • BOLD fMRI of brain tumors • BOLD fMRI studies of the brain, but neither comparing anaesthetic protocols nor investigating alterations of physiological parameters

	○ pharmacological ○ electrical ○ optogenetic • peripheral stimulation ○ electrical ○ mechanical ○ chemical ○ thermic ○ visual ○ auditory ○ olfactory ○ gustatory ○ visceral	
Definition of anaesthetics	• all inhalant anaesthetics (e.g. isoflurane, sevoflurane, halothane) • barbiturates (e.g. thiopental) • propofol, alfaxalone • ketamine, S-ketamine • α-chloralose • urethane • xylazine, medetomidine, dexmedetomidine • acepromazine • benzodiazepines • opioids if part of a balanced anaesthesia protocol	• opioids as sole sedative or as intervention in pain studies
Physiological parameters under investigation	• arterial blood pressure • heart rate • respiratory rate • pCO_2 • pO_2 • SpO_2 • pulse distension • body temperature • hematocrit	• all other parameters, e.g. blood glucose levels • local temperature of the brain • BOLD response to hyperoxia/hypercapnia as mere application to compare two groups
Interventions to alter physiological parameters	• blood withdrawal • fluid supplementation • pharmacologic manipulation of cardiovascular parameters • normobaric changes of inspiratory gas composition • apnoea • changes in body temperature	• hyperbaric inspiratory gas
Outcome	• any measure derived from	• correlations of BOLD signal

measures	BOLD signal alone	with other modalities (e.g. EEG signal)
Language restrictions	• English • German • French	• all other languages

6.4 Inclusion and exclusion decisions

6.4.1 Inclusions

- Boonzaier et al. (2017): fMRI, repeated transcranial magnetic stimulation under different anaesthetic protocols, then check with fMRI for changes in fc – and there were differences. Decision that only baseline data is included.
- Schmidt et al. (2006): clearly declare in the abstract that they want to analyse the contribution of the change of physiological parameters on fMRI activation and they do so by investigating the time course. As the fMRI signal changes persisted after HR, BP and RR returned to baseline, the authors conclude that the influence of PP changes is negligible in this case. This conclusion is clearly written in the abstract (results and discussion don't really comment on it that explicitly). The study separately reports BOLD, CBF and $CMRO_2$. Therefore, there is no reason to exclude it. The study was not classified as interventional study because transient hypercapnia served just to derive maps of the calibration parameter M.
- Huang et al. (2013): Hypercapnia used as an application, therefore this part of the study excluded (and also because no comment on whether the observed change was significant, only the difference in response pre/post methylene blue investigated for significance (no significant difference found)). However, the comparison of responses to paw stimulation under hypoxia versus normoxia is included.
- Vanhoutte et al. (2006): included because explicitly described what happens with whole brain BOLD signal intensity when body core temperature in-/decreased.
- Baskerville et al. (2011): characterizes T2* response to 40 vs 100 % oxygen in penumbra of ischemic lesion. Not considered an application of hyperoxia because they explicitly test if 40% is an alternative to 100% which causes oxygen artefacts from oxygen in the sinuses.
- Bock et al. (1998): paw stimulation at baseline, then a 3 min hypercapnia scan without paw stimulation, 25 min later another paw stimulation scan; no lasting effect of hypercapnia on BOLD response detected. Included because it shows that effects of transient hypercapnia are limited to the duration of actual hypercapnia, which is relevant for monitoring guidelines.
- Sedlacik et al. (2015): "Correlation of oxygenation and perfusion sensitive MRI with invasive micro probe measurements in healthy mice brain" uses different inspiratory gas concentrations and reports among many other outcomes R2*.
- Pan et al. (2011): "Interestingly, while increasing the level of isoflurane from 1% to 1.8%, correlated BOLD fluctuations between hemispheres as well as coherent theta/delta LFP power fluctuations increased significantly" was at first sight the only sentence about rsfMRI results and appears only in discussion part. However, the same finding was also reported in results: the authors defined a neural suppression index, based on amount of suppression in burst-suppression EEG, as a proxy for anaesthetic level and report correlation of BOLD fluctuations with that neural suppression index.
- Chen et al. (2008): included because results from figure in text described (summary from images in fig. 3), although the word "weaker" may be not

optimal – the images suggest that both less extended and lower signal change, but no quantitative analysis of % signal change between groups was provided.

6.4.2 Exclusions

6.4.2.1 Calibrated / quantitative fMRI without separate reporting of BOLD signal

- Wu et al. (2002): "Transient relationships among BOLD, CBV, and CBF changes in rat brain as detected by functional MRI". Aims of the study in its own words: 1. to experimentally determine how regional relationships between CBV and CBF fit the power law, 2. determine the transient relationships between CBF and CBV. The BOLD signal is separately described, but the article reports changes in BOLD signal during 7.5% and 10 % hypercapnia only in a graph, not in the text. It states that the displayed time courses were averages from those voxels that showed significant signal change during hypercapnia but does not report how many voxels did show a significant signal increase and how they were spatially distributed. Focus of the paper is on $CMRO_2$, parameter M, power index α, etc. Excluded as "insufficient detail of results" with the reason given here.
- Shu et al. (2016b): "Brain region and activity-dependent properties of M for calibrated fMRI". Compares medetomidine and α-chloralose only regarding parameter M, which is calculated as TE times R2' and thus not *sensu strictu* a BOLD measure (i.e. directly derived from signal intensity, R2* or T2*).
- Shu et al. (2016a): "Quantitative β mapping for calibrated fMRI"**.** Excluded because comparison of α-chloralose and medetomidine only regarding parameter β, which is part of a formula including $CMRO_2$. Signal intensity, T2* or R2* are not reported for the comparison of interest.

6.4.2.2 Application of hyperoxia or hypercapnia

- Kennan et al. (2004): application of hyperoxia in sickle cell model
- Elbel et al. (2000): abstract, considered an application of hypercapnia. Only the following sentence about hypercapnia: "By adding stepwise up to 10 percent CO_2 to the inspiratory gas mixture a generalized BOLD response was induced".
- Mahmoud et al. (2016): "AMP-activated Protein Kinase Deficiency Blocks the Hypoxic Ventilatory Response and Thus Precipitates Hypoventilation and Apnea" : Experimental hypoxia regarded as application (i.e. excluded) because of the following sentence: "Brainstem activity during hypoxia was therefore assessed by fMRI in anaesthetized mice in light of the fact that 9% or less O_2 induces cerebral vasodilation sufficient to eliminate the blood oxygen – level dependent (BOLD) signal driven, under normoxia, by increases in cerebral blood flow in response to sensory stimulation (27)".
- Ciobanu et al. (2015): uses hyperoxia as a "well known paradigm that mimics the BOLD response in absence of neuronal activation, by virtue of the

diamagnetic character gained by haemoglobin upon activation” to compare three different pulse sequences. Excluded as application of hyperoxia.

- Kim et al. (2014): hypercapnia as an application to test difference between 2 strains; focus on (correlation with) CBF and CBV; just one graph showing increase of BOLD signal during hypercapnia
- Shih et al. (2011): hypercapnic challenge mentioned in methods, but no results reported; probably as an application
- Mitschelen et al. (2009): hypercapnia "as a stimulus to probe the responsiveness of the vasculature" in adult, healthy aged and cognitively impaired aged rats
- Henninger et al. (2007): application of hypercapnia to compare groups after traumatic brain injury. Control animals „robust“ BOLD response to CO_2 (whole brain slice coloured in figure), after TBI “severely” reduced and different recovery rates between regions. Nevertheless, it’s an application, analogous to hyperoxia in sickle cell models.
- Paley et al. (2001): primarily application of hypercapnia, hyperoxia and hypoxia to test coherence of fMRI and optical imaging, additionally insufficient detail of results (no comment on significance of signal change). Focus of study on describing an MR device.
- Zhou et al. (2005): studies the interaction of magnetization transfer and BOLD effect : “The purpose of this study was to quantify the MTR changes in the brain as a function of arterial PCO_2 level and to use this dependence to study the interaction between the BOLD and MT effects in the parenchyma”. As declared in that sentence, variation of inspired CO_2 concentrations served primarily to characterize magnetization transfer. Accordingly, there is only one sentence in the results that the unsaturated BOLD signal increased by 7% during hypercapnia and that this increase was stronger in the saturated condition. Or in their words: “It can be seen that there is a change of about 7% in the unsaturated signal intensities (standard SE BOLD effect) but an increased magnitude up to approximately 15% for the signal intensities under off-resonance RF irradiation (combined MT BOLD and standard SE BOLD effect) corresponding to a 100 mm Hg change in PCO_2.” Excluded as application of hypercapnia and insufficient detail of results (no statement on significance).
- Wang et al. (2012): my understanding is that the “total” fMRI signal in this article is what is usually called the BOLD signal. It is known that other mechanisms contribute to the BOLD signal and what this study does is to investigate the contribution of perfusion or large vessel inflow to the total signal under hypercapnia, using hypercapnia as a method to increase CBF, i.e. an application. The focus is not on characterizing the response of the total signal to hypercapnia, but to elucidate the contributing mechanisms.

6.4.2.3 Anaesthetics not reported

- Williams et al. (2013): “Minocycline interferes with glutamate neurotransmission in an animal model of psychosis and with neurovascular coupling”. Administers ketamine on top, but anaesthetics used for imaging not reported. Additionally, characterising ketamine effects simply as causing “widespread activation” would fulfil the criteria for insufficient detail of results.

- Dunn et al. (1996): short form, clearly describes regional difference in response to hypoxia (cortex vs caudate putamen), but excluded because anaesthetics not reported.

6.4.2.4 No results for the comparison of interest

- He et al. (2008): investigates whether one can conclude from BOLD signal to oxygen saturation but doesn't describe how the BOLD signal differed between α-chloralose and isoflurane. Excluded as "no results reported for comparison of interest".
- Madularu et al. (2017)
- Pronger et al. (2014): "Mulitimodal neuroimaging with hypercapnia to monitor cerebrovascular function after traumatic brain injury and evaluate treatment". Only an abstract; compared isoflurane with medetomidine, but no results reported. All four databases and google scholar searched by name of first author for potential follow-up articles, but nothing found.
- Jones et al. (1996): values in a table plus a graph indicate that R2* increases during anoxia - I suppose that this must be a consistent and significant phenomenon, because the whole results and discussion section just compare the change of R2* during anoxia between ischemic and non-ischemic regions and between ischemia and reperfusion phases. However, as this study reports "numbers", but does not provide information about the statistical significance for the comparison of interest, and does not even descriptively summarize what happens to R2* in general during anoxia, it was excluded as "no results reported for the comparison of interest" and "insufficient detail of results".

6.4.2.5 Focus on vascular aspects

- Desjardins et al. (2014): focus on vascular aspects and correlation of BOLD with other modalities; only one graph depicting the time course of the BOLD signal after transient hypercapnia. Excluded as no results reported for the comparison of interest
- 1990 Ogawa et al. (1990): described changes apply to vessels, not parenchyma
- Ciobanu et al. (2012): vessels, not parenchyma imaged
- Uhrig et al. (2014): vessels, not parenchyma imaged

6.4.2.6 Insufficient detail of result only reason

- Wang et al. (2015): evaluates a method that measures BOLD and CBF concurrently. Exposed rats to hypercapnia and ischemia to test the method under disturbances. The BOLD signal is reported to increase under hypercapnia (4.5 %) and decrease under ischemia (23 %), however, whether these changes are significant is not reported. Further analysis of the BOLD signal only addresses correlation with other measures. Therefore, the study was excluded (reason: insufficient detail of results).
- Lahti et al. (1997): the subtraction is baseline image awake 1 minus baseline image awake 2 and NOT awake vs anaesthetized as assumed previously (see

methods section). Regarding the awake vs anaesthetized comparison, there is only one sentence in the methods: “The relatively large BOLD signal intensity change observed in this study may be due to the increased neuronal activation status of conscious animal, compared to the anesthetized counterpart.” This information comes without any further characterisation or assessment of significance and consequently the study was excluded as insufficient detail of results.

- Lin et al. (1999): ”A marked decrease in R2* was observed initially (from roughly 150 to 500 seconds) in response to the change in the CO_2 content of the inspired gas followed by a relative plateau throughout the end of the experiment. (…) Similar temporal behaviour for ΔR2* in response to hypercapnia was observed in all rats.” No comment on significance, therefore excluded as “insufficient detail”. The focus of this study is that you cannot derive cerebral blood oxygen saturation from ΔR2* because CBV has a strong effect. Authors describe method ad quantitative fMRI, but to me more similar to TRUST described in the textbook by Uludağ and Uğurbil (2015).

6.4.2.7 Singular reasons

- Nair and Duong (2004) and Ahrens and Dubowitz (2001) **placed the mice vertically in the scanner (vertical bore)**. Both studies were excluded because we considered haemodynamics in this position not comparable with haemodynamics in horizontal positioning.
- Morton et al. (2002): “Systemic theophylline augments the blood oxygen level-dependent response to forepaw stimulation in rats”. Used theophylline to inhibit cerebral vasodilation in response to neuronal activation and observed that the BOLD signal change during paw stimulation increased under theophylline while blood pressure and other systemic physiological parameters remained stable. As the aim of giving theophylline was explicitly not to modulate systemic physiological parameters, this intervention does not qualify as investigation of physiological parameter effects and the article was excluded.
- Two related articles:
    - Kettunen et al. (2001): “Cerebral T1ρ relaxation time increases immediately upon global ischemia in the rat independently of blood glucose and anoxic depolarization” was considered an “other MRI” earlier in screening stage 2 but would have been excluded anyway because of the lack of AP/PP.
    - Kettunen et al. (2002): “Effects of intracellular pH, blood, and tissue oxygen tension on T1ρ relaxation in rat brain” focuses as well on T1ρ as main MRI outcome, but uses different FiO_2 and $FiCO_2$. “In the in vivo experiments blood relaxation and cerebral blood volume (CBV) results were used for a two-compartment model to compute tissue T1 relaxation separately from the parenchymal MRI data.” In the results, there are two sentences about BOLD: “During hypoxia, a negative BOLD was evident as T2 was lowered by 3.3 +/- 0.6 ms.” And “the increase in T2 due to a BOLD effect was fully expressed at P_aCO_2 of around 80 mmHg, consistent with a previous cat study showing that

OER decrease stabilized at a hypercapnia level of around 80 mmHg". The rest of the text deals with T1rho. The study is therefore excluded as well and classified as "other MRI".

- Kipervaser et al. (2007): "Statistical framework and noise sensitivity of the amplitude radial correlation contrast method". Different anaesthetics (and concentrations) are used as a mean to test a statistical model. Excluded as other, reason: different states of anaesthesia just to test statistical model
- Plaschke et al. (2006): chronic effects of hypotension (as a method to induce transient cerebral oligemia) are investigated; however for imaging studies effects of acute hypotension are relevant
- Martin et al. (2009); Martin et al. (2013) (abstract and full article with same title): applied hypercapnia in awake animals → excluded because not a naturally occurring problem in awake animals
- Ogawa et al. (1993): "The venous blood oxygenation, monitored at the sagittal sinus with the blood water T, measurement, were varied by changing the depth of anaesthesia with halothane." → excluded as other. Changing the depth of anaesthesia is not an accepted method to modulate physiological parameters, because the effects of anaesthetic depth and change in oxygenation cannot be separated.
- Thomas et al. (2002): focus on describing a (back then new) imaging sequence, hypoxia only in 2 rats, some changes in T2(*) reported at the end of results section. Decrease of T2* during hypoxia; "reproducible" time course "changes are in the range 10 – 15 ms for T*2 and 5 – 10 ms for T2. This corresponds to a reduction in SpO_2 from 95% to a minimum value of approximately 60%". They "validate" their observations of T2* under hypoxia (5 episodes per animal, 2 animals) against one observation of T2* time course under normoxia. Excluded first because only one animal for control and second because no comment on significance, also not in methods or discussion (although I admit that it is probably reasonable NOT to use statistics here, but at some point I have to stick with defined criteria and this is clearly a quantitative outcome).
- Cash et al. (2003): 6 animals undergoing MRI, four "groups", but not clear how many animals per group or if maybe even crossover. Excluded because realistic chance that n=1 per condition. "one animal per condition" selected as reason of exclusion.
- Easton et al. (2009): does not fulfil definition of an observational physiological parameter study, because intention to investigate effects of systemic physiological parameters on BOLD signal not expressed in abstract. But even if it would: title and axis are not matching in graphs so that it is not clear which graph shows what and results are not interpretable!

6.4.2.8 Abstract collections

Abstract collections are explicitly labelled as such in the title and, if an "abstract" is displayed in DistillerSR, it typically states something like "This proceeding contains 476 papers". Abstract collections were not manually screened but excluded *in toto*.

- Abstracts from the 11th Congress of the European Association of Neuro-Oncology (2014)

- 11th Turkish Neuroscience Congress (2013)
- 3rd Biennial Conference on Resting State Brain Connectivity(2012)
- Abstracts of the 20th Congress of the European Sleep Research Society (2010)
- Abstracts and Programme - EUROANAESTHESIA 2009: The European Anaesthesiology Congress (2009c)
- 9th International Symposium on NeuroVirology (2009a)
- 2011 5th International IEEE/EMBS Conference on Neural Engineering, NER 2011 (2011)
- 13th International Conference on Biomedical Engineering, ICBME 2008
- (2009b)

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6.5 List of definitively included references

Reference ID	Authors	Title	Year
13122	Schroeter, A.,Grandjean, J.,Schlegel, F.,Saab, B. J.,Rudin, M.	Contributions of structural connectivity and cerebrovascular parameters to functional magnetic resonance imaging signals in mice at rest and during sensory paw stimulation	2017
13123	Smith, J. B.,Liang, Z.,Watson, G. D. R.,Alloway, K. D.,Zhang, N.	Interhemispheric resting-state functional connectivity of the claustrum in the awake and anesthetized states	2017
13136	Grandjean, J.,Preti, M. G.,Bolton, T. A. W.,Buerge, M.,Seifritz, E.,Pryce, C. R.,Van De Ville, D.,Rudin, M.	Dynamic reorganization of intrinsic functional networks in the mouse brain	2017
13142	Abe, Y.,Tsurugizawa, T.,Le Bihan, D.	Water diffusion closely reveals neural activity status in rat brain loci affected by anesthesia	2017
13145	Wu, T.,Grandjean, J.,Bosshard, S. C.,Rudin, M.,Reutens, D.,Jiang, T.	Altered regional connectivity reflecting effects of different anaesthesia protocols in the mouse brain	2017
13158	Hamilton, C.,Ma, Y.,Zhang, N.	Global reduction of information exchange during anesthetic-induced unconsciousness	2017
13169	Bukhari, Q.,Schroeter, A.,Cole, D. M.,Rudin, M.	Resting state fMRI in mice reveals anesthesia specific signatures of brain functional networks and their interactions	2017
13170	Ma, Y.,Hamilton, C.,Zhang, N.	Dynamic Connectivity Patterns in Conscious and Unconscious Brain	2017
13184	Brynildsen, J. K.,Hsu, L. M.,Ross, T. J.,Stein, E. A.,Yang, Y.,Lu, H.	Physiological characterization of a robust survival rodent fMRI method	2017
13186	Gill, R. S.,Mirsattari, S. M.,Leung, L. S.	Resting state functional network disruptions in a kainic acid model of temporal lobe epilepsy	2017
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13199	Tsurugizawa, T.,Takahashi, Y.,Kato, F.	Distinct effects of isoflurane on basal BOLD signals in tissue/vascular microstructures in rats	2016

13203	Yoshida, K.,Mimura, Y.,Ishihara, R.,Nishida, H.,Komaki, Y.,Minakuchi, T.,Tsurugizawa, T.,Mimura, M.,Okano, H.,Tanaka, K. F.,Takata, N.	Physiological effects of a habituation procedure for functional MRI in awake mice using a cryogenic radiofrequency probe	2016
13234	Hudetz, A. G.,Liu, X.,Pillay, S.,Boly, M.,Tononi, G.	Propofol anesthesia reduces Lempel-Ziv complexity of spontaneous brain activity in rats	2016
13242	Chang, P. C.,Procissi, D.,Bao, Q.,Centeno, M. V.,Baria, A.,Apkarian, A. V.	Novel method for functional brain imaging in awake minimally restrained rats	2016
13247	Shah, D.,Blockx, I.,Keliris, G. A.,Kara, F.,Jonckers, E.,Verhoye, M.,Van der Linden, A.	Cholinergic and serotonergic modulations differentially affect large-scale functional networks in the mouse brain	2016
13265	Zhurakovskaya, E.,Paasonen, J.,Shatillo, A.,Lipponen, A.,Salo, R.,Aliev, R.,Tanila, H.,Gröhn, O.	Global functional connectivity differences between sleep-like states in urethane anesthetized rats measured by fMRI	2016
13282	Paasonen, J.,Salo, R. A.,Shatillo, A.,Forsberg, M. M.,Närväinen, J.,Huttunen, J. K.,Gröhn, O.	Comparison of seven different anesthesia protocols for nicotine pharmacologic magnetic resonance imaging in rat	2016
13309	Paasonen, J.,Salo, R. A.,Huttunen, J. K.,Gröhn, O.	Resting-state functional MRI as a tool for evaluating brain hemodynamic responsiveness to external stimuli in rats	2016
13345	Grimm, O.,Gass, N.,Weber-Fahr, W.,Sartorius, A.,Schenker, E.,Spedding, M.,Risterucci, C.,Schweiger, J. I.,Böhringer, A.,Zang, Z.,Tost, H.,Schwarz, A. J.,Meyer-Lindenberg, A.	Acute ketamine challenge increases resting state prefrontal-hippocampal connectivity in both humans and rats	2015
13361	Liang, Z.,Watson, G. D. R.,Alloway, K. D.,Lee, G.,Neuberger, T.,Zhang, N.	Mapping the functional network of medial prefrontal cortex by combining optogenetics and fMRI in awake rats	2015

13362	Nasrallah, F. A.,Yeow, L. Y.,Biswal, B.,Chuang, K. H.	Dependence of BOLD signal fluctuation on arterial blood CO<inf>2</inf> and O<inf>2</inf>: Implication for resting-state functional connectivity	2015
13364	Schlegel, F.,Schroeter, A.,Rudin, M.	The hemodynamic response to somatosensory stimulation in mice depends on the anesthetic used: Implications on analysis of mouse fMRI data	2015
13374	Bettinardi, R. G.,Tort-Colet, N.,Ruiz-Mejias, M.,Sanchez-Vives, M. V.,Deco, G.	Gradual emergence of spontaneous correlated brain activity during fading of general anesthesia in rats: Evidences from fMRI and local field potentials	2015
13399	Hudetz, A. G.,Liu, X.,Pillay, S.	Dynamic repertoire of intrinsic brain states is reduced in propofol-induced unconsciousness	2015
13403	Lai, H. Y.,Albaugh, D. L.,Kao, Y. C. J.,Younce, J. R.,Shih, Y. Y. I.	Robust deep brain stimulation functional MRI procedures in rats and mice using an MR-compatible tungsten microwire electrode	2015
13422	Liang, Z.,Liu, X.,Zhang, N.	Dynamic resting state functional connectivity in awake and anesthetized rodents	2015
13464	Nasrallah, F. A.,Low, S. M. A.,Lew, S. K.,Chen, K.,Chuang, K. H.	Pharmacological insight into neurotransmission origins of resting-state functional connectivity: α2-adrenergic agonist vs antagonist	2014
13471	Grandjean, J.,Schroeter, A.,Batata, I.,Rudin, M.	Optimization of anesthesia protocol for resting-state fMRI in mice based on differential effects of anesthetics on functional connectivity patterns	2014
13472	Kundu, P.,Santin, M. D.,Bandettini, P. A.,Bullmore, E. T.,Petiet, A.	Differentiating BOLD and non-BOLD signals in fMRI time series from anesthetized rats using multi-echo EPI at 11.7T	2014
13482	Gong, L.,Li, B.,Wu, R.,Li, A.,Xu, F.	Brain-state dependent uncoupling of BOLD and local field potentials in laminar olfactory bulb	2014
13493	Schroeter, A.,Schlegel, F.,Seuwen, A.,Grandjean, J.,Rudin, M.	Specificity of stimulus-evoked fMRI responses in the mouse: The influence of systemic physiological changes associated with innocuous stimulation under four different anesthetics	2014
13500	Chao, T. H. H.,Chen, J. H.,Yen, C. T.	Repeated BOLD-fMRI imaging of deep brain stimulation responses in rats	2014

13523	Magnuson, M. E.,Thompson, G. J.,Pan, W. J.,Keilholz, S. D.	Time-dependent effects of isoflurane and dexmedetomidine on functional connectivity, spectral characteristics, and spatial distribution of spontaneous BOLD fluctuations	2014
13525	Gass, N.,Schwarz, A. J.,Sartorius, A.,Schenker, E.,Risterucci, C.,Spedding, M.,Zheng, L.,Meyer-Lindenberg, A.,Weber-Fahr, W.	Sub-anesthetic ketamine modulates intrinsic BOLD connectivity within the hippocampal-prefrontal circuit in the rat	2014
13533	Nasrallah, F. A.,Tay, H. C.,Chuang, K. H.	Detection of functional connectivity in the resting mouse brain	2014
13542	Jonckers, E.,Palacios, R. D.,Shah, D.,Guglielmetti, C.,Verhoye, M.,Van Der Linden, A.	Different anesthesia regimes modulate the functional connectivity outcome in mice	2014
13547	Nasrallah, F. A.,Lew, S. K.,Low, A. S. M.,Chuang, K. H.	Neural correlate of resting-state functional connectivity under α2 adrenergic receptor agonist, medetomidine	2014
13634	Liu, X.,Pillay, S.,Li, R.,Vizuete, J. A.,Pechman, K. R.,Schmainda, K. M.,Hudetz, A. G.	Multiphasic modification of intrinsic functional connectivity of the rat brain during increasing levels of propofol	2013
13637	Liang, Z.,Li, T.,King, J.,Zhang, N.	Mapping thalamocortical networks in rat brain using resting-state functional connectivity	2013
13703	Liu, X.,Zhu, X. H.,Zhang, Y.,Chen, W.	The change of functional connectivity specificity in rats under various anesthesia levels and its neural origin	2013
13715	Kalthoff, D.,Po, C.,Wiedermann, D.,Hoehn, M.	Reliability and spatial specificity of rat brain sensorimotor functional connectivity networks are superior under sedation compared with general anesthesia	2013
13729	Huang, S.,Du, F.,Shih, Y. Y. I.,Shen, Q.,Gonzalez-Lima, F.,Duong, T. Q.	Methylene blue potentiates stimulus-evoked fMRI responses and cerebral oxygen consumption during normoxia and hypoxia	2013
13841	Liu, X.,Li, R.,Yang, Z.,Hudetz, A. G.,Li, S. J.	Differential effect of isoflurane, medetomidine, and urethane on BOLD responses to acute levo-tetrahydropalmatine in the rat	2012
13843	Liang, Z.,King, J.,Zhang, N.	Intrinsic organization of the anesthetized brain	2012

13849	Airaksinen, A. M.,Hekmatyar, S. K.,Jerome, N.,Niskanen, J. P.,Huttunen, J. K.,Pitkänen, A.,Kauppinen, R. A.,Gröhn, O. H.	Simultaneous BOLD fMRI and local field potential measurements during kainic acid-induced seizures	2012
13890	Nasrallah, F. A.,Tan, J.,Chuang, K. H.	Pharmacological modulation of functional connectivity: α 2-adrenergic receptor agonist alters synchrony but not neural activation	2012
13891	Sumiyoshi, A.,Suzuki, H.,Ogawa, T.,Riera, J. J.,Shimokawa, H.,Kawashima, R.	Coupling between gamma oscillation and fMRI signal in the rat somatosensory cortex: Its dependence on systemic physiological parameters	2012
13903	Liang, Z.,King, J.,Zhang, N.	Anticorrelated resting-state functional connectivity in awake rat brain	2012
13911	Tu, Y.,Yu, T.,Fu, X. Y.,Xie, P.,Lu, S.,Huang, X. Q.,Gong, Q. Y.	Altered thalamocortical functional connectivity by propofol anesthesia in rats	2011
13930	Herman, P.,Sanganahalli, B. G.,Hyder, F.,Eke, A.	Fractal analysis of spontaneous fluctuations of the BOLD signal in rat brain	2011
13948	Pan, W. J.,Thompson, G.,Magnuson, M.,Majeed, W.,Jaeger, D.,Keilholz, S.	Broadband Local Field Potentials Correlate with Spontaneous Fluctuations in Functional Magnetic Resonance Imaging Signals in the Rat Somatosensory Cortex Under Isoflurane Anesthesia	2011
13959	Wilson, D. A.,Hoptman, M. J.,Gerum, S. V.,Guilfoyle, D. N.	State-dependent functional connectivity of rat olfactory system assessed by fMRI	2011
13976	Min, D. K.,Tuor, U. I.,Chelikani, P. K.	Gastric distention induced functional magnetic resonance signal changes in the rodent brain	2011
13988	Desai, M.,Kahn, I.,Knoblich, U.,Bernstein, J.,Atallah, H.,Yang, A.,Kopell, N.,Buckner, R. L.,Graybiel, A. M.,Moore, C. I.,Boyden, E. S.	Mapping brain networks in awake mice using combined optical neural control and fMRI	2011
13995	de Celis Alonso, B.,Makarova, T.,Hess, A.	On the use of α-chloralose for repeated BOLD fMRI measurements in rats	2011

13996	Kalthoff, D.,Seehafer, J. U.,Po, C.,Wiedermann, D.,Hoehn, M.	Functional connectivity in the rat at 11.7T: Impact of physiological noise in resting state fMRI	2011
13999	Liu, X.,Zhu, X. H.,Zhang, Y.,Chen, W.	Neural origin of spontaneous hemodynamic fluctuations in rats under burst-suppression anesthesia condition	2011
14009	Wang, K.,Van Meer, M. P.,Van Der Marel, K.,Van Der Toorn, A.,Xu, L.,Liu, Y.,Viergever, M. A.,Jiang, T.,Dijkhuizen, R. M.	Temporal scaling properties and spatial synchronization of spontaneous blood oxygenation level-dependent (BOLD) signal fluctuations in rat sensorimotor network at different levels of isoflurane anesthesia	2011
14036	Williams, K. A.,Magnuson, M.,Majeed, W.,LaConte, S. M.,Peltier, S. J.,Hu, X.,Keilholz, S. D.	Comparison of α-chloralose, medetomidine and isoflurane anesthesia for functional connectivity mapping in the rat	2010
14056	Hutchison, R. M.,Mirsattari, S. M.,Jones, C. K.,Gati, J. S.,Leung, L. S.	Functional networks in the anesthetized rat brain revealed by independent component analysis of resting-state fMRI	2010
14084	Tsurugizawa, T.,Uematsu, A.,Uneyama, H.,Torii, K.	Effects of isoflurane and alpha-chloralose anesthesia on BOLD fMRI responses to ingested l-glutamate in rats	2010
14121	Sommers, M. G.,van Egmond, J.,Booij, L. H. D. J.,Heerschap, A.	Isoflurane anesthesia is a valuable alternative for α-chloralose anesthesia in the forepaw stimulation model in rats	2009
14131	Pawela, C. P.,Biswal, B. B.,Hudetz, A. G.,Schulte, M. L.,Li, R.,Jones, S. R.,Cho, Y. R.,Matloub, H. S.,Hyde, J. S.	A protocol for use of medetomidine anesthesia in rats for extended studies using task-induced BOLD contrast and resting-state functional connectivity	2009
14144	Lu, J.,Dai, G.,Egi, Y.,Huang, S.,Kwon, S. J.,Lo, E. H.,Kim, Y. R.	Characterization of cerebrovascular responses to hyperoxia and hypercapnia using MRI in rat	2009
14199	Asanuma, T.,Yasui, H.,Sato, M.,Inanami, O.,Kuwabara, M.	A BOLD-fMRI study of cerebral activation induced by injection of algesic chemical substances into the anesthetized rat forepaw	2008
14219	Kannurpatti, S. S.,Biswal, B. B.,Kim, Y. R.,Rosen, B. R.	Spatio-temporal characteristics of low-frequency BOLD signal fluctuations in isoflurane-anesthetized rat brain	2008

14234	Chen, C. M.,Shih, Y. Y. I.,Siow, T. Y.,Chiang, Y. C.,Chang, C.,Jaw, F. S.	Antinociceptive effect of morphine in α-chloralose and isoflurane anesthetized rats using bold fMRI	2008
14238	Huttunen, J. K.,Gröhn, O.,Penttonen, M.	Coupling between simultaneously recorded BOLD response and neuronal activity in the rat somatosensory cortex	2008
14246	Maandag, N. J. G.,Coman, D.,Sanganahalli, B. G.,Herman, P.,Smith, A. J.,Blumenfeld, H.,Shulman, R. G.,Hyder, F.	Energetics of neuronal signaling and fMRI activity	2007
14258	Herman, P.,Sanganahalli, B.,Hyder, F.,Eke, A.	Non-invasive hypotensive FMRI study in rat somatosensory cortex	2007
14268	Tuor, U. I.,Wang, R.,Zhao, Z.,Foniok, T.,Rushforth, D.,Wamsteeker, J. I.,Qiao, M.	Transient hypertension concurrent with forepaw stimulation enhances functional MRI responsiveness in infarct and peri-infarct regions	2007
14304	Qiao, M.,Rushforth, D.,Wang, R.,Shaw, R. A.,Tomanek, B.,Dunn, J. F.,Tuor, U. I.	Blood-oxygen-level-dependent magnetic resonance signal and cerebral oxygenation responses to brain activation are enhanced by concurrent transient hypertension in rats	2007
14321	Duong, T. Q.	Cerebral blood flow and BOLD fMRI responses to hypoxia in awake and anesthetized rats	2007
14363	Littlewood, C. L.,Cash, D.,Dixon, A. L.,Dix, S. L.,White, C. T.,O'Neill, M. J.,Tricklebank, M.,Williams, S. C. R.	Using the BOLD MR signal to differentiate the stereoisomers of ketamine in the rat	2006
14377	Wang, R.,Foniok, T.,Wamsteeker, J. I.,Qiao, M.,Tomanek, B.,Vivanco, R. A.,Tuor, U. I.	Transient blood pressure changes affect the functional magnetic resonance imaging detection of cerebral activation	2006
14378	Schmidt, K. F.,Febo, M.,Shen, Q.,Luo, F.,Sicard, K. M.,Ferris, C. F.,Stein, E. A.,Duong, T. Q.	Hemodynamic and metabolic changes induced by cocaine in anesthetized rat observed with multimodal functional MRI	2006
14381	Vanhoutte, G.,Verhoye, M.,Van Der Linden, A.	Changing body temperature affects the T2* signal in the rat brain and reveals hypothalamic activity	2006

14382	Littlewood, C. L.,Jones, N.,O'Neill, M. J.,Mitchell, S. N.,Tricklebank, M.,Williams, S. C. R.	Mapping the central effects of ketamine in the rat using pharmacological MRI	2006
14388	Weber, R.,Ramos-Cabrer, P.,Wiedermann, D.,Van Camp, N.,Hoehn, M.	A fully noninvasive and robust experimental protocol for longitudinal fMRI studies in the rat	2006
14401	Kuo, C. C.,Chen, J. H.,Tsai, C. Y.,Liang, K. C.,Yen, C. T.	BOLD signals correlate with ensemble unit activities in rat's somatosensory cortex	2005
14409	Ramos-Cabrer, P.,Weber, R.,Wiedermann, D.,Hoehn, M.	Continuous noninvasive monitoring of transcutaneous blood gases for a stable and persistent BOLD contrast in fMRI studies in the rat	2005
14423	Kalisch, R.,Delfino, M.,Murer, M. G.,Auer, D. P.	The phenylephrine blood pressure clamp in pharmacologic magnetic resonance imaging: Reduction of systemic confounds and improved detectability of drug-induced BOLD signal changes	2005
14437	Sicard, K. M.,Duong, T. Q.	Effects of hypoxia, hyperoxia, and hypercapnia on baseline and stimulus-evoked BOLD, CBF, and CMRO2 in spontaneously breathing animals	2005
14445	Dashti, M.,Geso, M.,Williams, J.	The effects of anaesthesia on cortical stimulation in rats: A functional MRI study	2005
14459	Austin, V. C.,Blamire, A. M.,Allers, K. A.,Sharp, T.,Styles, P.,Matthews, P. M.,Sibson, N. R.	Confounding effects of anesthesia on functional activation in rodent brain: A study of halothane and α-chloralose anesthesia	2005
14502	Kannurpatti, S. S.,Biswal, B. B.	Effect of anesthesia on CBF, MAP and fMRI-BOLD signal in response to apnea	2004
14533	Brevard, M. E.,Duong, T. Q.,King, J. A.,Ferris, C. F.	Changes in MRI signal intensity during hypercapnic challenge under conscious and anesthetized conditions	2003
14539	Tenney, J. R.,Duong, T. Q.,King, J. A.,Ludwig, R.,Ferris, C. F.	Corticothalamic modulation during absence seizures in rats: A functional MRI assessment	2003
14544	Kannurpatti, S. S.,Biswal, B. B.,Hudetz, A. G.	Baseline physiological state and the fMRI-BOLD signal response to apnea in anesthetized rats	2003
14548	Kannurpatti, S. S.,Biswal, B. B.,Hudetz, A. G.	Regional dynamics of the fMRI-BOLD signal response to hypoxia-hypercapnia in the rat brain	2003

14558	Sicard, K.,Shen, Q.,Brevard, M. E.,Sullivan, R.,Ferris, C. F.,King, J. A.,Duong, T. Q.	Regional cerebral blood flow and BOLD responses in conscious and anesthetized rats under basal and hypercapnic conditions: Implications for functional MRI studies	2003
14562	Luo, F.,Wu, G.,Li, Z.,Li, S. J.	Characterization of effects of mean arterial blood pressure induced by cocaine and cocaine methiodide on bold signals in rat brain	2003
14569	Tuor, U. I.,McKenzie, E.,Tomanek, B.	Functional magnetic resonance imaging of tonic pain and vasopressor effects in rats	2002
14584	Dutka, M. V.,Scanley, B. E.,Does, M. D.,Gore, J. C.	Changes in CBF-BOLD coupling detected by MRI during and after repeated transient hypercapnia in rat	2002
14589	Kannurpatti,	Erratum: Differential fMRI-BOLD signal response to apnea in humans and anesthetized rats (Magnetic Ressonance in Medicine (2002) 47:5 (864-870))	2002
14615	Kalisch, R.,Elbel, G. K.,Gössl, C.,Czisch, M.,Auer, D. P.	Blood pressure changes induced by arterial blood withdrawal influence bold signal in anesthesized rats at 7 Tesla: Implications for pharmacologic MRI	2001
14617	Peeters, R. R.,Tindemans, I.,De Schutter, E.,Van der Linden, A.	Comparing BOLD fMRI signal changes in the awake and anesthetized rat during electrical forepaw stimulation	2001
14654	Xu, H.,Li, S. J.,Bodurka, J.,Zhao, X.,Xi, Z. X.,Stein, E. A.	Heroin-induced neuronal activation in rat brain assessed by functional MRI	2000
14670	Zaharchuk, G.,Mandeville, J. B.,Bogdanov Jr, A. A.,Weissleder, R.,Rosen, B. R.,Marota, J. J. A.	Cerebrovascular dynamics of autoregulation and hypoperfusion: An MRI study of CBF and changes in total and microvascular cerebral blood volume during hemorrhagic hypotension	1999
14678	Dunn, J. F.,Zaim Wadghiri, Y.,Meyerand, M. E.	Regional heterogeneity in the brain's response to hypoxia measured using BOLD MR imaging	1999
14685	Hempel, E.,Reith, W.,Elste, V.,Heiland, S.,Sartor, K.	Influence of stimulus frequency, amplitude and blood pressure on signal change in fMRI	1999
14688	Lahti, K. M.,Ferris, C. F.,Li, F.,Sotak, C. H.,King, J. A.	Comparison of evoked cortical activity in conscious and propofol-anesthetized rats using functional MRI	1999
14696	Lin, W.,Paczynski, R. P.,Celik, A.,Hsu, C.	Effects of acute normovolemic hemodilution on T2*-weighted images	1998

	Y.,Powers, W. J.	of rat brain	
14702	Hsu, E. W.,Hedlund, L. W.,MacFall, J. R.	Functional MRI of the rat somatosensory cortex: Effects of hyperventilation	1998
14703	Lin, W.,Paczynski, R. P.,Celik, A.,Hsu, C. Y.,Powers, W. J.	Experimental hypoxemic hypoxia: Effects of variation in hematocrit on magnetic resonance T2*-weighted brain images	1998
14715	Bock, C.,Schmitz, B.,Kerskens, C. M.,Gyngell, M. L.,Hossmann, K. A.,Hoehn-Berlage, M.	Functional MRI of somatosensory activation in rat: Effect of hypercapnic up-regulation on perfusion- and BOLD-imaging	1998
14716	Lin, W.,Paczynski, R. P.,Celik, A.,Kuppusamy, K.,Hsu, C. Y.,Powers, W. J.	Experimental hypoxemic hypoxia: Changes in R2* of brain parenchyma accurately reflect the combined effects of changes in arterial and cerebral venous oxygen saturation	1998
14721	Dunn, J. F.,Swartz, H. M.	Blood oxygenation: Heterogeneity of hypoxic tissues monitored using bold MR imaging	1997
14734	Kida, I.,Yamamoto, T.,Tamura, M.	Interpretation of BOLD MRI signals in rat brain using simultaneously measured near-infrared spectrophotometric information	1996
14746	Graham, G. D.,Zhong, J.,Petroff, O. A. C.,Constable, R. T.,Prichard, J. W.,Gore, J. C.	BOLD MRI monitoring of changes in cerebral perfusion induced by acetazolamide and hypercarbia in the rat	1994
15189	Sedlacik, J.,Reitz, M.,Bolar, D. S.,Adalsteinsson, E.,Schmidt, N. O.,Fiehler, J.	Correlation of oxygenation and perfusion sensitive MRI with invasive micro probe measurements in healthy mice brain	2015
15316	Mechling, A. E.,Hubner, N. S.,Lee, H. L.,Hennig, J.,von Elverfeldt, D.,Harsan, L. A.	Fine-grained mapping of mouse brain functional connectivity with resting-state fMRI	2014
15923	Lowry, J. P.,Griffin, K.,McHugh, S. B.,Lowe, A. S.,Tricklebank, M.,Sibson, N. R.	Real-time electrochemical monitoring of brain tissue oxygen: A surrogate for functional magnetic resonance imaging in rodents	2010
16784	Houston, G. C.,Papadakis, N. G.,Carpenter, T. A.,Hall, L. D.,Mukherjee, B.,James, M. F.,Huang,	Mapping of the cerebral response to hypoxia measured using graded asymmetric spin echo EPI	2000

	C. L. H.		
20115	Baskerville, T. A.,Deuchar, G. A.,McCabe, C.,Robertson, C. A.,Holmes, W. M.,Santosh, C.,Macrae, I. M.	Influence of 100% and 40% oxygen on penumbral blood flow, oxygen level, and T2 -weighted MRI in a rat stroke model	2011
22637	Prielmeier, F.,Nagatomo, Y.,Frahm, J.	Cerebral blood oxygenation in rat brain during hypoxic hypoxia. Quantitative MRI of effective transverse relaxation rates	1994
22791	Prielmeier, F.,Merboldt, K. D.,Hanicke, W.,Frahm, J.	Dynamic high-resolution MR imaging of brain deoxygenation during transient anoxia in the anesthetized rat	1993
23151	Boonzaier, J.,Van Tilborg, G. A. F.,Straathof, M.,Petrov, P. I.,Van Heijningen, C. L.,Van Vliet, G.,Smirnov, N.,Van Der Toorn, A.,Neggers, S. F.,Dijkhuizen, R. M.	Differential outcomes of rTMS and anesthesia effects on functional connectivity in the rat brain	2017
24362	Sanganahalli, B. G.,Herman, P.,Blumenfeld, H.,Hyder, F.	FMRI and electrophysiological studies with α-chloralose and domitor anesthesia	2009
29730495	HJ Shim, WB Jung, F Schlegel, J Lee, S Kim, J Lee, SG Kim	Mouse fMRI under ketamine and xylazine anesthesia: Robust contralateral somatosensory cortex activation in response to forepaw stimulation.	2018
29730496	W. Gsell, A. Giarola, T. Reese, A. J. Schwarz , H. Barjat, S. Smart, A. Gozzi, S. Bertani , V. Crestan, A. Bifone	Carry-over effects of gaseous anaesthesia on fMRI response and tissue oxygen levels in the rat brain	n.d.

6.6 The adapted SYRCLE risk of bias tool

Questions can be answered with “yes”, indicating a low risk of bias, “no”, indicating a high risk of bias, and “unclear”, meaning that the risk of bias is unclear based on the information provided in the respective publication. Guidelines of all journals in which the included references are published were checked for specific requirements regarding blinding and randomization (list see below). None of those journals claimed to publish only randomized or blinded studies.

To summarize the risk of bias per study as well as across studies, the approach proposed in the Cochrane risk of bias tool will be used (Higgins et al., 2011).

6.6.1 Items

1. **Selection bias – sequence generation**

   Describe the methods used, if any, to generate the allocation sequence in sufficient detail to allow an assessment whether it should produce comparable groups.

   Was the allocation sequence adequately generated and applied?

   Did the investigators describe a random component in the sequence generation process such as:
   Referring to a random number table;
   Using a computer random number generator.

   Additional information

   Examples of a non-random approach:
   - Allocation by judgment or by investigator’s preference;
   - Allocation based on the results of a laboratory test or a series of tests;
   - Allocation by availability of the intervention;
   - Sequence generated by odd or even date of birth;
   - Sequence generated by some rule based on animal number or cage number.

   Practical implementation
   - If method described → yes or no depending on method
   - If method not described → unclear

2. **Selection bias – baseline characteristics**
   Describe animal characteristics, if any, that are compared in order to judge whether or not intervention and control groups were similar at the start of the experiment.

Were the groups similar at baseline or were they adjusted for confounders in the analysis?

Was the distribution of relevant baseline characteristics balanced for the intervention and control groups?

If relevant, did the investigators adequately adjust for unequal distribution of some relevant baseline characteristics in the analysis?

Additional information

Relevant baseline characteristics are age, sex and weigth; timepoint of baseline image acquisition relative to induction and eventual change of anaesthetic; response to stimulation at baseline.

Practical implementation

- If clearly similar → yes
- If partially similar, but not all relevant information reported → unclear
- If clearly different or no information on any of the relevant characteristics reported → no

3. Selection bias – allocation concealment

Describe the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen before or during enrolment.

Was the allocation adequately concealed?

Could the investigator allocating the animals to intervention or control group not foresee assignment due to one of the following or equivalent methods?

- Third-party coding of experimental and control group allocation
- Central randomization by a third party
- Sequentially numbered opaque, sealed envelopes

Additional information

Examples of investigators allocating the animals being possibly able to foresee assignments:

- Open randomization schedule
- Envelopes without appropriate safeguard
- Alternation or rotation
- Allocation based on date of birth
- Allocation based on animal number
- Any other explicitly unconcealed procedure of a non-random approach

Practical implementation

- If method described → yes or no according to list of positive and negative examples
- If method not described → unclear

4. Performance bias – blinding

Describe all measures used, if any, to blind trial caregivers and researchers from knowing which intervention each animal received. Provide any information relating to whether the intended blinding was effective.

Were the caregivers and /or investigators blinded from knowledge which intervention each animal received during the experiment? Were animals selected at random for outcome assessment?

Was blinding of caregivers and investigators ensured, and was it unlikely that their blinding could have been broken?

- ID cards of individual animals, or cage/animal labels are coded and identical in appearance.
- Administration of anaesthetics is performed by a person not involved in image processing and outcome assessment; the investigators cannot see the procedure and the vaporizer setting.
- The circumstances during the intervention are specified and similar in both groups.

Additional information

Examples of inappropriate blinding:

- Colored cage labels (red for group A, yellow group B)
- Expected differences in visible effects between control and experimental groups
- The individual who prepares the experiment is the same as the one who conducts and analyses the experiment
- Circumstances during the intervention are not similar in both groups
- Examples where circumstances during the intervention were not similar:
- Timing of administration of the placebo and exp drug was different; timing of imaging different between different groups
- Instruments used to conduct experiment differ between experimental and control group (e.g. skinning of skull for optical imaging or electrode insertion into the brain in one group, but not the other)

Practical implementation

As blinding is associated with additional work for the investigators and increases the level of evidence ascribed to a study, we expected that any effort to blind a study is at least briefly mentioned (e.g. “a blinded investigator”).

- If blinding described and expected to have been effective → yes
- If blinding mentioned, but not described how/doubts whether effective → unclear
- If no mention of blinding at all → no
- If for one aspect of the experiment clear that not blinded → no, because very unlikely that rest blinded

5. Detection bias – random outcome assessment

Describe whether or not animals were selected at random for outcome assessment, and which methods to select the animals, if any, were used.

Were animals selected at random for outcome assessment?

Did the investigators randomly pick an animal during outcome assessment, or did they use a random component in the sequence generation for outcome assessment?

- Referring to a random number table;
- Using a computer random number generator;
- Etc

Practical implementation

- If method described → yes or no depending on method
- If method not described → unclear

6. Detection bias – blinding

Describe all measures used, if any, to blind outcome assessors from knowing which intervention each animal received. Provide any information relating to whether the intended blinding was effective.

Was the outcome assessor blinded?

Was blinding of the outcome assessor ensured, and was it unlikely that blinding could have been broken?

- Outcome assessment methods were the same in
- both groups.
- Animals were selected at random during outcome
- assessment (use signaling questions of entry 6).

Was the outcome assessor not blinded, but do review authors judge that the outcome is not likely to be influenced by lack of blinding? (e.g., mortality)

Additional information

This item needs to be assessed for each main outcome.

Practical implementation
As blinding is associated with additional work for the investigators and increases the level of evidence ascribed to a study, we expected that any effort to blind a study is at least briefly mentioned (e.g. “a blinded investigator”).

- If blinding described and expected to have been effective → yes
- If blinding mentioned, but not described how/doubts whether effective → unclear
- If no mention of blinding at all → no
- If for one aspect of the experiment clear that not blinded → no, because very unlikely that rest blinded

7. Attrition bias – incomplete outcome data

Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attritions and exclusions were reported, the numbers in each intervention group (compared with total randomized animals), reasons for attrition or exclusions, and any re-inclusions in analyses for the review.

Were incomplete data adequately addressed?

Were all animals included in the analysis?

Were the reasons for missing outcome data unlikely to be related to true outcome (e.g., technical failure)?
Are missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups?

Practical implementation

- If clearly stated that all animals were included → yes
- If neither stated that all animals included nor exclusions reported or if missing outcome data not balanced across groups → unclear
- If missing suspicion that exclusions related to true outcome or results reported for fewer animals than originally included without justification → no

8. Reporting bias – selective outcome reporting

State how selective outcome reporting was examined and what was found.

Are reports of the study free of selective outcome reporting?

Was the study protocol available and were all of the study's pre-specified primary and secondary outcomes reported in the current manuscript?

Was the study protocol not available, but was it clear that the published report included all expected outcomes (i.e. comparing methods and results section)?

Additional information

Selective outcome reporting:

- Not all of the study' s pre-specified primary outcomes have been reported;
- One or more primary outcomes have been reported using measurements, analysis methods or data subsets (e.g., subscales) that were not pre-specified in the protocol;
- One or more reported primary outcomes were not pre-specified (unless clear justification for their reporting has been provided, such as an unexpected
- adverse effect);
- The study report fails to include results for a key outcome that would be expected to have been reported for such a study.

Practical implementation

If no study protocol was available, the second question was decisive for the overall assessment of item 8.

- If no suspicion → yes
- If not sure → unclear
- If expected results were not reported → no

9. Other – other source of bias

State any important concerns about bias not covered by other domains in the tool.

Was the study apparently free of other problems that could result in a high risk of bias?

Was the study free of pooling drugs?

Additional information

Experiments in which animals receive – besides the intervention drug – additional treatment or drugs which might influence or bias the result. Eg. administration of inhalant anaesthetic together with N2O, but injectable anaesthetic administered without adding N2O or administration of injectable anaesthetic associated with fluid administration, but no fluids administered in inhalant anaesthetic group.

Were design-specific risks of bias absent?

Additional information

Design-specific risks of bias:

- Crossover design that was not suitable (intervention with no temporary effect, or the disease is not stable over time)
- Crossover design with risk of carry-over effect
- Crossover design with only first period data being available
- Crossover design with many animals not receiving 2nd or following treatment due to large number of drop-outs probably due to longer duration of study
- Crossover design in which all animals received same order of interventions
- Multi-arm study in which the same comparisons of groups are not reported for all outcomes (selective outcome reporting)
- Multi-arm study in which results of different arms are combined (all data should be presented per group)
- Cluster randomized trial not taking clustering into account during statistical analysis (unit of analysis error)
- Crossover design in which paired analysis of the results is not taken into account

Practical implementation

- If pooling of drugs explicitly avoided and no concern about design-specific risk of bias → yes
- If pooling of drugs or design-specific risk of bias possible → unclear
- If pooling of drugs (e.g. if one group inhalants and other group injectables and fluid management not described) or clear design-specific risk of bias → no

6.6.2 Check of author guidelines

Journals for which author guidelines were checked and no specific requirements regarding blinding/randomization standards were found:

- Australasian Physical and Engineering Sciences in Medicine
- Biomedical Engineering - Applications, Basis and Communications
- Brain Connectivity
- Brain Research
- Brain Stimulation
- Brain Structure and Function
- Brain Topography
- Cerebral Cortex
- Chinese Journal of Physiology
- Epilepsia
- European Neuropsychopharmacology
- Frontiers in Neural Circuits
- Japanese Journal of Veterinary Research
- Journal of Cerebral Blood Flow & Metabolism
- Journal of Magnetic Resonance Imaging
- Journal of Neurophysiology
- Journal of Neuroscience
- Journal of Neuroscience Methods
- Magnetic Resonance Imaging
- Magnetic Resonance in Medicine
- NeuroImage.
- NeuroImage: Clinical
- Neuropsychopharmacology
- NeuroReport
- Neuroscience
- Neuroscience Letters
- NMR in Biomedicine
- Pharmacology
- PLoS Biology
- PLOSone
- Proceedings of the National Academy of Sciences of the United States of America
- Psychopharmacology
- RoFo Fortschritte auf dem Gebiete der Rontgenstrahlen und der Neuen Bildgebenden Verfahren
- Scientific Reports
- Stroke
- Zeitschrift Fur Medizinische Physik

Journals for which author guidelines were not found or not in English

- Advances in Experimental Medicine and Biology
- Chirurgia Italiana

References

Higgins, J.P.T., Altman, D.G., Gøtzsche, P.C., Jüni, P., Moher, D., Oxman, A.D., Savović, J., Schulz, K.F., Weeks, L., Sterne, J.A.C., 2011. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ 343.

6.7 Forms for screening and data extraction

6.7.1 Title abstract screening

Question Text	Type	Answer Text
Should reference go on to full text screening?	Radio	yes
		no
		can't tell
Reason of exclusion	Checkbox	other species
		other region of body
		other fMRI modality
		review
		completely different method
		out of age range
		in vitro
		book chapter
		commentary/opinion article
		model description
		data processing
		planned or ongoing study
Keep a personal copy?	Checkbox	yes

6.7.2 Full text screening

Question Text	Type	Answer Text
Method:	Radio	BOLD fMRI
		other MRI
		no MRI
Location:	Radio	brain
		brain tumor only (e.g. glioma)
		other body region
Species:	Checkbox	rat
		mouse
		other
Age group:	Radio	adult
		younger
		older
Type of publication:	Radio	short form
		full article
		review/opinion piece/talk
Other reasons for exclusion present?	Checkbox	duplicate

		multiple reporting
		full article available
		baseline study
		one animal per condition
		anesthetic(s) not reported
		no results reported for the comparison of interest
		imaging conditions different between conditions
		insufficient detail of results
		other
		no
Comparison of anesthetic states?	Checkbox	not relevant (already excluded)
		different drugs
		different dosages
		different timepoints
		awake vs anesthetized
		no
Changes in physiological parameters?	Checkbox	not relevant (already excluded)
		experimentally induced
		naturally occuring, explicitely monitored
		no
This reference will be:	Radio	Included
		Excluded
Keep a personal copy?	Checkbox	yes

6.7.3 Study characteristics

Question Text	**Type**	**Answer Text**
rat strain	Checkbox	Wistar
		Sprague-Dawley
		Long-Evans
		na
mouse strain	Checkbox	C57BL/6
		BALB/c
		I/LnJ
		transgenic ChR2
sex	Checkbox	male
		female
		na
comments strain/sex	Text	
age (average +/- sd or range in	Text	

weeks)		
weight (average +/- sd or range in grams)	Text	
total number of animals	Text	
number of animals undergoing fMRI	Text	
Invasive procedures during prep (other than art/ven catheterization)?	Checkbox	craniotomy (to expose a certain area)
		thinning of skull (to create a window)
		electrode insertion into the brain (with or without small drill holes)
		tracheostomy
		-
		na
		Attachment of a head-bar to the skull
		exposing skull
		epidural electrode insertion
		gastric tube
		Implanted with intra-gastric (IG) balloons or IG catheters
		middle cerebral artery occlusion
Study Design	Checkbox	comparison between groups (one exp.session)
		comparison to baseline (same baseline for all animals, same experimental session)
		comparison to earlier session (same first measurement for all animals, multiple sessions))
		crossover - one session
		crossover - multiple sessions
		comparison of time points of measurement
		observation
		not clear (na)
study design - free text	Text	
experimental paradigm	Checkbox	peripheral stimulation
		central stimulation
		resting state
		PPi as stimulus (i.e. signal pre-/post-PPi analyzed as with other stimuli)

		calibrated
		other
peripheral stimulation	Checkbox	electrical (somatosensory)
		mechanical (somatosensory)
		chemical (somatosensory)
		thermal (somatosensory)
		whisker stimulation
		olfactory
		gustatory
		auditory
		visual
		visceral - mechanical
		visceral - chemical (e.g. ingestion of nutrients)
central stimulation	Checkbox	electrical
		chemical/pharmacological
		optogenetic
PPi as stimulus	Checkbox	change in breathing gas mixture
		apnea
		spontanoeus vs. controlled ventilation
		blood withdrawal
		fluid supplementation
		pharmacologic (cardiovascular parameters)
		change in body temperature
Comment on stimulation	Text	
field strength in Tesla	Text	
time points and duration of image acquisition	Text	
ROIs in this study	Text	
general approach to fMRI data	Text	
analysis of PP data (if applicable, otherwise just â€œ-â€œ)	Text	

6.7.4 Anaesthetic protocol

Question Text	**Type**	**Answer Text**
AP experimental group name - drug	Radio	isoflurane
		sevoflurane
		halothane
		iso/medet
		iso/dexmedet

		medetomidine
		dexmedetomidine
		ketamin/xylazin
		ketamin/medetomidine
		ketamine/dexmedet
		a-choralose
		urethane
		propofol
		Pentobarbital
		plus ketamine
		plus s-ketamine
		plus r-ketamine
		ketamine - xylazine - acepromazine
		ketamine/xylazine/acepromazine
		thiobutabarbital
		equithesine
		iso plus ketamine/xylazine
		N2O
		iso plus ketamine
		iso plus medet
		midazolam
AP experimental group name - dose	Radio	low dose
		intermediate dose
		high dose
		short iso
		long iso
		spont vent
		contr vent
		after awake
		without awake
induction	Checkbox	inhalation
		injection
		na
inhalant induc	Radio	isoflurane
		sevoflurane
		halothane
		isoflurane or halothane
vaporizer setting (%) induc	Text	
method inhalant induc	Radio	box
		mask
		na
total gas flow (l/min) induc	Text	
gas mixture (in %) induc	Checkbox	oxygen

		air
		nitrogen N2
		nitrous oxide N2O
		na
injectable 1 induc	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
		chloralhydrate
		Pentobarbital
		equithesine
		atropine
dose/unit/route injectable 1 induc	Text	
injectable 2 induc	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
		-
dose/unit/route injectable 2 induc	Text	
Airway management	Radio	intubation
		nose cone/mask
		na
	Radio	oral
		tracheotomy
		na
Ventilation mode	Radio	spontaneous
		controlled
		na
Maintenance during preparation and set-up	Checkbox	inhalation
		CRI
		top-ups
		induction = maintenance

		na
inhalant prep	Radio	isoflurane
		sevoflurane
		halothane
		isoflurane or halothane
vaporizer setting (%) prep	Text	
if stop inhalant after prep: timepoint in min after initial bolus/start CRI maintenance exp.	Text	
CRI 1 prep	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
initial bolus CRI 1 prep (dose/unit/route)	Text	
rate CRI 1 prep (dose/unit/route)	Text	
timing CRI 1 prep (start CRI relativ to induction/bolus; eventual stop)	Text	
CRI 2 prep	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
		-
initial bolus CRI 2 prep (dose/unit/route)	Text	
rate CRI 2 prep (dose/unit/route)	Text	
timing CRI 2 prep (start CRI relativ to induction/bolus; eventual stop)	Text	
top-up 1 prep	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
		Pentobarbital
unit/dose/route top-up 1 prep	Text	

interval top-up 1 prep (repeated after x min)	Text	
top-up 2 prep	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
		-
unit/dose/route top-up 2 prep	Text	
interval top-up 2 prep (repeated after x min)	Text	
total gas flow (l/min) prep	Text	
gas mixture (in %) prep	Checkbox	oxygen
		air
		nitrogen N2
		nitrous oxide N2O
		na
Maintenance during experiment	Checkbox	inhalation
		CRI
		top-ups
		induction = maintenance
inhalant exp	Radio	isoflurane
		sevoflurane
		halothane
		N2O
vaporizer setting (%) exp	Text	
CRI 1 exp	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
		midazolam
initial bolus CRI 1 exp (dose/unit/route)	Text	
rate CRI 1 exp (dose/unit/route)	Text	
timing CRI 1 exp (start CRI relativ to initial bolus/stop inhalant/...)	Text	

rate CRI 1 exp after change	Text	
timing rate change CRI 1 exp and additional information	Text	
CRI 2 exp	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
		-
initial bolus CRI 2 exp (dose/unit/route)	Text	
rate CRI 2 exp (dose/unit/route)	Text	
timing CRI 2 exp (start CRI relativ to initial bolus/stop inhalant/..)	Text	
rate CRI 2 exp after change	Text	
timing rate change CRI 2 exp and additional information	Text	
top-up 1 exp	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
		Pentobarbital
		acepromazine
		thiobutabarbital
		equithesine
		midazolam
unit/dose/route top-up 1 exp	Text	
interval top-up 1 exp (repeated after x min)	Text	
top-up 2 exp	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane

		-
unit/dose/route top-up 2 exp	Text	
interval top-up 2 exp (repeated after x min)	Text	
total gas flow (l/min) exp	Text	
gas mixture (in %) exp	Checkbox	oxygen
		air
		nitrogen N2
		nitrous oxide N2O
		na
Was an anesthetic drug given on top of the maintenance protocol as an experimental stimulus?	Radio	yes
		no
on top - drug	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		r-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
		heroin
on top - doses/unit/routes/timing	Text	
Local anesthesia used?	Radio	yes
		no
site of local anesthetic	Text	
local anesthetic	Radio	lidocaine
		ropivacaine
		bupivacaine
		mepivacaine
dose/unit local anesthetic	Text	
Animals paralyzed?	Radio	yes
		no
NMBA	Checkbox	pancuronium bromide
		gallamine
		mivacurium
		D-tubocurarine chloride

		na
dose/unit/route NMBA	Text	
Other drugs routinely administered (name, dose, route, timepoint/interval)	Text	
Comments/explanations anesthetic protocol	Text	

6.7.5 Awake protocol

Question Text	Type	Answer Text
awake group name	Radio	awake
Was an acclimatization protocol used prior to imaging?	Radio	yes
		no
Describe the acclimatization protocol:	Text	
Were the animals anesthetized for placement in the scanner (in the actual imaging session)?	Radio	yes
		no
induction	Checkbox	inhalation
		injection
inhalant induc	Radio	isoflurane
		sevoflurane
		halothane
vaporizer setting (%) induc	Text	
method inhalant induc	Radio	box
		mask
		na
total gas flow (l/min) induc	Text	
gas mixture (in %) induc	Checkbox	oxygen
		air
		nitrogen N2
		nitrous oxide N2O
		na
injectable 1 induc	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol

		alfaxalone
		a-chloralose
		urethane
		chloralhydrate
dose/unit/route injectable 1 induc	Text	
injectable 2 induc	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
		-
dose/unit/route injectable 2 induc	Text	
Maintenance during preparation and set-up	Checkbox	inhalation
		CRI
		top-ups
		induction = maintenance
inhalant prep	Radio	isoflurane
		sevoflurane
		halothane
vaporizer setting (%) prep	Text	
CRI 1 prep	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
initial bolus CRI 1 prep (dose/unit/route)	Text	
rate CRI 1 prep (dose/unit/route)	Text	
timing CRI 1 prep (start CRI relativ to induction/bolus; eventual stop)	Text	
CRI 2 prep	Radio	medetomidine
		dexmedetomidine
		xylazine

		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
		-
initial bolus CRI 2 prep (dose/unit/route)	Text	
rate CRI 2 prep (dose/unit/route)	Text	
timing CRI 2 prep (start CRI relativ to induction/bolus; eventual stop)	Text	
top-up 1 prep	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
unit/dose/route top-up 1 prep	Text	
interval top-up 1 prep (repeated after x min)	Text	
top-up 2 prep	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
		-
unit/dose/route top-up 2 prep	Text	
interval top-up 2 prep (repeated after x min)	Text	
total gas flow (l/min) prep	Text	
gas mixture (in %) prep	Checkbox	oxygen
		air
		nitrogen N2
		nitrous oxide N2O
		na
total gas flow (l/min) exp	Text	
gas mixture (in %) exp	Checkbox	oxygen
		air
		nitrogen N2
		nitrous oxide

		N2O
		na
Was an anesthetic drug given in subanesthetic dose as an experimental stimulus?	Radio	yes
		no
on top - drug	Radio	medetomidine
		dexmedetomidine
		xylazine
		ketamine
		s-ketamine
		propofol
		alfaxalone
		a-chloralose
		urethane
on top - doses/unit/routes/timing	Text	
Local anesthesia used?	Radio	yes
		no
site of local anesthetic	Text	
local anesthetic	Radio	lidocaine
		ropivacaine
		bupivacaine
		mepivacaine
dose/unit local anesthetic	Text	
Animals paralyzed?	Radio	yes
		no
NMBA	Radio	pancuronium bromide
		mivacurium
dose/unit/route NMBA	Text	
Comments/explanations awake protocol	Text	

6.7.6 Monitoring

Question Text	**Type**	**Answer Text**
Type of experimental groups	Radio	fMRI anesthetized
		bench-top
		fMRI awake
Monitored parameters	Checkbox	body temperature
		heart rate
		arterial blood pressure
		respiratory rate
		respiratory pattern
		respiratory gases (Fi/Fe)
		SpO2/S(a/v)O2

		pO2
		pCO2
		pH of the blood
		reflexes
		pulse distension (in um)
		hemoglobin concentration
		concentration of volatile agents (Fi and/or Fe)
		Fi/Fe N2O
		hematocrit
		glucose
temperature kept at (average +/- sd or range in Â°C)	Text	
heart rate - method of measurement	Checkbox	pulseoxymetry
		arterial line (invasive BP)
		ECG
		tail pulse cuff sensor
		not specified
blood pressure - method of measurement	Checkbox	invasive
		noninvasive
respiratory rate - method of measurement	Checkbox	pressure sensitive sensor
		piezo electric force transducer
		capnography
		spirometry
		ECG
		visual (observing the animal)
		not specified
		controlled ventilation
		artline
respiratory pattern - definition and method of measurement	Text	
respiratory gases	Checkbox	FiO2
		FeO2
		FiCO2
		FeCO2
SpO2/S(a/v)O2 - method of measurement	Checkbox	pulsoxymetry
		arterial blood gas
		venous blood gas
		transcutaneous
		not specified
pO2 - method of measurement	Checkbox	arterial blood gas
		venous blood gas

		transcutaneous
		not specified
pCO2 method of measurement	Checkbox	arterial blood gas
		venous blood gas
		transcutaneous
		not specified
pH - method of measurement	Checkbox	arterial blood gas
		venous blood gas
		not specified
reflexes tested	Checkbox	tail flick
		hind limb withdrawal
		not specified
		whiskering
		â€œblinkâ€œ
		forelimb withdrawal
		tail pinch
		corneal
		palpebral reflex
		righting reflex
Timepoints of blood gas sampling and/or reflex testing	Text	
Comments on the monitoring used (eg relevant method for analysis, details controlled ventilation)	Text	

6.7.7 Physiological parameters interventions

Question Text	Type	Answer Text
Name of phys.p.i. experimental group	Radio	hypotension
		hypertension
		hypoxia
		hyperoxia
		hypocapnia
		hypercapnia
		apnoea - room air baseline
		apnoea - hypoxic baseline
		apnoea - hyperoxic baseline
		apnoea - hypocapnic baseline
		apnoea - hypercapnic baseline

		apnoea - hyperoxic, hypercapnic baseline
		hypothermia
		hyperthermia
		anemia
		tachycardia
		hyperoxia-hypercapnia
		variation of body temperature
		Room air with apnoea
		100% O2 with apnoea
		Apnoea with carbogen (5% CO2 + 95% O2)
		2% CO2 + 98% air with apnea
		5% CO2 + 95% air with apnoea
		all gas mixes
		Spontaneous vent. vs mechanical vent.
		contr vent
		spont vent
		variable BP
		constant BP
Name of phys.p.i. experimental group - stimulus strength	Radio	strongest
		intermediate
		weakest
		urethane
		pentobarbital
		2%, urethane
		2%, pentobarbital
		5%, urethane
		5%, pentobarbital
		awake
		iso
		normal hct
		mild anemia
		moderate anemia
Type of intervention	Checkbox	change in breathing gas mixture
		apnoea
		blood withdrawal
		fluid supplementation
		pharmacologic
		change in body temperature

		spontaneous vs controlled ventilation
		change in controlled ventilation settings
		negative lower body pressure
breathing gas mix baseline	Checkbox	oxygen
		air
		N2
		N2O
		CO2
		na
breathing gas mix stimulus	Checkbox	oxygen
		air
		N2
		N2o
		CO2
		na
breathing gas mix post stimulus	Checkbox	oxygen
		air
		N2
		N20
		CO2
		na
apnoea - duration (in s)	Text	
apnoea - number of cycles	Text	
apnoea - interval between cycles	Text	
blood withdrawal - amount in ml (per step)	Text	
blood withdrawal - over time (per step)	Text	
blood withdrawal - number of steps	Text	
blood withdrawal - total amount	Text	
blood withdrawal - total time	Text	
fluid supplementation - fluid type	Checkbox	cristalloids
		colloids
		blood
fluid supplmentation - amount in ml per bolus	Text	
fluid supplementation - time per bolus	Text	
fluid supplementation - number of boli	Text	
fluid supplementation - total amount	Text	
fluid supplementation - total time	Text	
pharmacologic - type of drug	Checkbox	inotrope
		vasopressor
		vasodilator

		other
pharmacologic - name of drug	Text	
pharmacologic - dose/unit/route	Text	
Parameters under investigation	Checkbox	body temperature
		heart rate
		arterial blood pressure
		respiratory rate
		respiratory pattern
		respiratory gases - EtCO2
		SpO2 / S(a/v)O2
		pO2
		pCO2
		pH of the blood
		hematocrit
		pulse distension
		other parameters, please specify
heart rate - direction of change	Checkbox	up
		down
		constant
		other analysis
heart rate - baseline (average +/- sd in bpm)	Text	
heart rate - under stimulation (average +/- sd)	Text	
heart rate - post stimulation (average +/- sd)	Text	
blood pressure - direction of change	Checkbox	up
		down
		constant
		other analysis
blood pressure - baseline (group average +/- sd in cmH2O)	Text	
blood pressure - under stimulation (average +/- sd)	Text	
blood pressure - post stimulation (average +/- sd)	Text	
pCO2 - direction of change	Checkbox	up
		down
		constant
		other analysis
pCO2 - baseline (group average +/- sd in mmHg)	Text	
pCO2 - under stimulation (average +/- sd)	Text	
pCO2 - post stimulation (average +/- sd)	Text	
pO2 - direction of change	Checkbox	up
		down

		constant
		other analysis
pO2 - baseline (group average +/- sd in mmHg)	Text	
pO2 - under stimulation (average +/- sd)	Text	
pO2 - post stimulation (average +/- sd)	Text	
SpO2 - direction of change	Checkbox	up
		down
		constant
		other analysis
SpO2 - baseline (group average +/- sd in %)	Text	
SpO2 - under stimulation (average +/- sd)	Text	
SpO2 - post stimulation (average +/- sd)	Text	
respiratory rate - direction of change	Checkbox	up
		down
		constant
		other analysis
respiratory rate - baseline (group average +/- sd in /min)	Text	
respiratory rate - under stimulation (average +/- sd)	Text	
respiratory rate - post stimulation (average +/- sd)	Text	
hematocrit - direction of change	Checkbox	up
		down
		constant
		other analysis
hematocrit - baseline (group average +/- sd in %)	Text	
hematocrit - under stimulation (average +/- sd)	Text	
hematocrit - post stimulation (average)	Text	
body temperature - direction of change	Checkbox	up
		down
		constant
		other analysis
body temperature - baseline (group average in Â°C)	Text	
body temperature - under stimulation (average +/- sd)	Text	
body temperature - post stimulation (average +/- sd)	Text	
pulse distension - direction of change	Checkbox	up
		down
		constant
		other analysis

pulse distension - baseline (average in um +/- sd)	Text	
pulse distension - under stimulation (average +/- sd)	Text	
pulse distension - post stimulation (average +/- sd)	Text	
respiratory pattern - type of change	Text	
Comments phys.p. int. anesth	Text	
ph of blood - direction of change	Checkbox	up
		down
		constant
		other analysis
ph of blood - baseline (average +/- sd)	Text	
ph of blood - under stimulation (average +/- sd)	Text	
pH of blood - post stimulation (average +/- sd)	Text	
EtCO2 - direction of change	Checkbox	up
		down
		constant
		other analysis
EtCO2 - baseline (average +/- sd)	Text	
EtCO2 - under stimulation (average +/- sd)	Text	
EtCO2 - post stimulation(average +/- sd)	Text	

6.7.8 Physiological parameters observations

Question Text	Type	Answer Text
Name of phys.p.obs. experimental group	Radio	PP observation
		PPo iso
		PPo medet
		PPo propofol
		PPo urethane
		PPo ac
		PPo ketamin/medet
Parameters under investigation	Checkbox	heart rate
		arterial blood pressure
		pH
		pCO2
		pO2
		SpO2

		hematocrit
		respiratory rate
		body temperature
		pulse distension
		other parameters, please specify
		glucose
heart rate - direction of change	Checkbox	up
		down
		constant
		other analysis
heart rate - baseline (average +/- sd in bpm)	Text	
heart rate - under stimulation (average +/- sd)	Text	
heart rate - post stimulation (average +/- sd)	Text	
blood pressure - direction of change	Checkbox	up
		down
		constant
		other analysis
blood pressure - baseline (group average +/- sd in cmH2O)	Text	
blood pressure - under stimulation (average +/- sd)	Text	
blood pressure - post stimulation (average +/- sd)	Text	
pH - direction of change	Checkbox	up
		down
		constant
		other analysis
pH - baseline (group average +/- sd)	Text	
pH - under stimulation (average +/- sd)	Text	
pH - post stimulation (average +/- sd)	Text	
pCO2 - direction of change	Checkbox	up
		down
		constant
		other analysis
pCO2 - baseline (group average +/- sd in mmHg)	Text	
pCO2 - under stimulation (average +/- sd)	Text	
pCO2 - post stimulation (average +/- sd)	Text	
pO2 - direction of change	Checkbox	up
		down
		constant

		other analysis
pO2 - baseline (group average +/- sd in mmHg)	Text	
pO2 - under stimulation (average +/- sd)	Text	
pO2 - post stimulation (average +/- sd)	Text	
SpO2 - direction of change	Checkbox	up
		down
		constant
		other analysis
SpO2 - baseline (group average +/- sd in %)	Text	
SpO2 - under stimulation (average +/- sd)	Text	
SpO2 - post stimulation (average +/- sd)	Text	
respiratory rate - direction of change	Checkbox	up
		down
		constant
		other analysis
respiratory rate - baseline (group average +/- sd in /min)	Text	
respiratory rate - under stimulation (average +/- sd)	Text	
respiratory rate - post stimulation (average +/- sd)	Text	
hematocrit - direction of change	Checkbox	up
		down
		constant
		other analysis
hematocrit - baseline (group average +/- sd in %)	Text	
hematocrit - under stimulation (average +/- sd)	Text	
hematocrit - post stimulation (average)	Text	
body temperature - direction of change	Checkbox	up
		down
		constant
		other analysis
body temperature - baseline (group average +/- sd in °C)	Text	
body temperature - under stimulation (average +/- sd)	Text	
body temperature - post stimulation (average +/- sd)	Text	
pulse distension - direction of change	Checkbox	up
		down
		constant
		other analysis
pulse distension - baseline (group	Text	

average +/- sd in um)		
pulse distension - stimulation (group average +/- sd in um)	Text	
pulse distension - post stimulation (group average +/- sd in um)	Text	
Comments PP obs anesth	Text	

6.7.9 Outcome anaesthetic protocols

Question Text	Type	Answer Text
Was a difference observed between different states of anesthesia?	Radio	yes
		no
Specify the observed difference(s)	Text	
In which measures was no difference observed?	Text	
Personal comments	Text	

6.7.10 Outcome physiological parameters

Question Text	Type	Answer Text
Was a difference observed between different physiological states?	Radio	yes
		no
Specify the observed difference(s)	Text	
In which measures was no difference observed?	Text	
Personal comments	Text	

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